DEPARTMENT OF THE AIR FORCE
AIR FORCE RESEARCH LABORATORY
WRIGHT-PATTERSON AIR FORCE BASE OHIO 45433

20 December 2001

MEMORANDUM FOR US EPA
NCEA (MD-52)
RTP, NC 27711
ATTN: ANNIE M. JARABEK

FROM: Elaine A. Merrill
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SUBJECT: Consultative Letter, AFRL-HE-WP-CL-2002-0003, Revision to AFRL-HE-WP-CL-2001-0010, Comparison of Internal Dosimetrics Using PBPK Models for Perchlorate-Induced Inhibition of Thyroid Iodide Uptake and Sensitivity Analysis for Male Rat Model.

1. This letter supercedes Consultative Letter, AFRL-HE-WP-CL-2001-0010 and provides corrected tables. The corrected reports are provided in Attachments 1 through 3 with the original cover letter in Attachment 4.

2. Changes include corrections to Table 11 in Attachment 1 and Table 11 in Attachment 2. In addition, a new table, providing ratios of percent inhibition between species and reproductive states was included as Table 9B in Attachment 1.

3. This letter describes the use of four physiologically based pharmacokinetic (PBPK) models to develop dosimetric measures of perchlorate pharmacokinetics. The perchlorate exposure scenarios simulated are serum and thyroid perchlorate concentrations and percent inhibition of iodide uptake into the thyroid after *iv* dosing in the male rat. These same dosimetrics were derived from drinking water exposures in male rat and pregnant and lactating rat as well as drinking water exposures in human subjects. Based on dosimetrics in the male rat, human equivalent exposure doses (HEEDs) were predicted.

4. A PBPK model parameter sensitivity analysis was performed on the male rat model with *iv* dosing at 0.1 and 1.0 mg/kg. All PBPK parameters controlling perchlorate kinetics were individually varied by one percent and the effect of each parameter change on the area under the perchlorate concentration curve in the serum and thyroid was used as a measure of model parameter sensitivity.

5. For further information, please contact me by phone: (937) 255-5150 ext. 3195, fax: (937) 255-1474 or e-mail: elaine.merrill@wpafb.af.mil.

Elaine A. Merrill
Operational Toxicology Branch

Attachments

1. Comparison of Internal Dosimetrics Using PBPK Models for Perchlorate Induced Inhibition of Thyroid Iodide Uptake and Sensitivity Analysis for Male Rat Model
2. PBPK-Derived Internal Dosimetrics
3. Statistics for Michaelis-Menten fits for Different Data Sets Represented in Figures 1 through 8
4. Consultative Letter, AFRL-HE-WP-CL-2001-0010, Comparison of Internal Dosimetrics Using PBPK Models for Perchlorate-Induced Inhibition of Thyroid Iodide Uptake and Sensitivity Analysis for Male Rat Model (original cover letter only).

1st Ind, AFRL/HEST 20 December 2001

MEMORANDUM FOR US EPA
ATTN: MS. ANNIE JARABEK

This letter report has been coordinated at the branch level and is approved for release.

David R. Mattie, PhD, DABT
Acting Branch Chief
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Human Effectiveness Directorate

Comparison of Internal Dosimetrics Using PBPK Models for Perchlorate Induced Inhibition of Thyroid Iodide Uptake and Sensitivity Analysis for Male Rat Model

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3 October 2001

Attachment 1

INTRODUCTION

Recently, perchlorate (ClO_4^-) has been found in surface and ground waters of various environmental sites, especially in the western United States (Urbansky, 1998). Due to the experimentally and clinically proven effectiveness of ClO_4^- to cause inhibition of iodide uptake in the thyroid gland (Wolff, 1998), concern has arisen over the potential health consequences to humans consuming waters containing ClO_4^- and to animals in the environment who might be exposed to this chemical.

One set of tools that is available to help quantitate the potential risk to humans and animals from ClO_4^- exposure is the use of physiologically based pharmacokinetic (PBPK) models. PBPK models provide a means of calculating the absorption, distribution, metabolism and excretion of a chemical in an organism and thereby provide a key element of classic toxicology, the dose in the dose-response relationship. With PBPK models, it is possible to estimate the mass of chemical in the blood and tissues of an exposed organism, which can then be correlated to the toxicological effect.

One of the most important applications of PBPK modeling is interspecies extrapolation. Since physiological constants of different species are known, parameter values for laboratory animals and humans can be used in the PBPK model structures to account for physiological differences between species. Once there is appropriate calibration and validating of a PBPK model, it is then possible to estimate ClO_4^- concentrations in blood and tissues of the rat and the human, under varying exposure situations.

This consultative letter will present the results of using four PBPK ClO_4^- models to calculate dosimetrics for ClO_4^- dosing of humans and male, pregnant, lactating, fetal and neonatal rats, correlated with inhibition of thyroidal uptake of iodide (Merrill *et al.*, 2001a and b; Clewell *et al.*, 2001a and b). The calculated dosimetrics include the area under the ClO_4^- time-concentration curve (AUC) in the serum and thyroid of rats and humans dosed with ammonium perchlorate, the total urinary excretion in a 24 hour period, peak serum and thyroid concentrations and the percent inhibition of iodide uptake into the thyroid. These dosimetrics are chosen to provide insight on interspecies extrapolation for various health effect endpoints at different life stages.

In addition to calculating dosimetrics for ClO_4^- exposure effects using the four PBPK models, a sensitivity analysis was also performed on the male rat model (Merrill *et al.*, 2001a), at *iv* doses of 0.1 and 1.0 mg/kg, to determine which parameters had the most significant impact on the two dosimetrics chosen. The *iv* dose route was chosen for model sensitivity analysis since acute rat inhibition response was considered the primary dose response effect for dosimetric reference. This analysis helps determine which model parameters have the most significant impact on tissue dose and, therefore, are most important to the risk assessment calculation. Accuracy of the risk assessment depends on the level of uncertainty of these most critical parameters. Determination of the most sensitive parameters in the model also provides a means for choosing which parameters should be the focus of future experimental efforts.

METHODS

The following internal dosimetrics were chosen to represent output from each of the PBPK models: area under the curve (AUC) for serum and thyroid ClO_4^- concentrations, peak serum and thyroid ClO_4^- concentrations, total amount of ClO_4^- excreted in the urine, AUC for the lactational and placental transfer of ClO_4^- and the percent inhibition of iodide uptake into the thyroid. In order to explore the dose-response relationship of these values, the target dosimetrics were evaluated across several doses in both acute and sub-chronic exposure scenarios using previously developed PBPK models for the male rat (Merrill *et al.*, 2001a), pregnant and fetal rats (Clewell *et al.*, 2001a) and lactating and neonatal rats (Clewell *et al.*, 2001b), as well as the adult (non-pregnant) human (Merrill *et al.*, 2001b). The PBPK models successfully describe perchlorate, iodide and inhibition data in acute *iv* and drinking water studies from our laboratory and in published literature.

In 1998, the EPA proposed a conceptual framework for the assessment of perchlorate and noted that both the potential cancer and noncancer effects are due to perturbations in the hypothalamic-pituitary-thyroid axis that result from iodide uptake inhibition at the Na^+/I^- symporter (NIS). Since even transient decrements in thyroxine (T_4) and triiodothyronine (T_3) may result in permanent neurodevelopmental defects, the most appropriate dosimetric would reflect these temporary hormone changes. Tumors, however, are more likely the result of chronic stimulation of the thyroid by TSH.

Although the most serious effect of iodide deficiency is delayed physical and mental development (Delange, 2000; Haddow *et al.*, 1999), brain tissue ClO_4^- concentration is not included as an internal dosimetric. The referenced developmental PBPK models (Clewell *et al.*, 2001a and b) do not contain brain compartments for perchlorate distribution since little ClO_4^- is able to pass into the brain (Yu *et al.*, 2000a). It is expected that diminished availability of T_4 and subsequent decreased concentration of T_3 in the brain of the fetus or infant is responsible for any observed effects.

Dosimetrics in Rat Models

Acute *iv* pharmacokinetic studies in the male rat were used as the basis for this dose-response analysis because iodide uptake inhibition could be correlated to perchlorate levels. In drinking water studies, up-regulation of NIS resulted in no measurable thyroid iodide inhibition. Therefore, the initial inhibition of iodide uptake represents the first in a series of events that leading to potential adverse health effects (e.g., hypothyroidism, thyroid tumors and possible neurodevelopmental disorders). The target internal dosimetrics were first calculated in each of the rat models for acute exposure to ClO_4^- (single *iv* administration) at doses of 0.01, 0.1, 1.0, 3.0, 5.0, 10.0, 30.0 and 100.0 mg/kg. In order to correlate ClO_4^- parameters to data-validated inhibition, the 2 to 4 hr time frame was used for all acute calculations. The AUC for thyroid and serum were calculated by integrating predicted tissue concentrations from 2 to 4 hrs post-dosing.

The same dosimetrics calculated for acute exposures were also determined for sub-chronic ClO_4^- exposures (drinking water) at doses of 0.01, 0.1, 1.0, 3.0, 5.0, 10.0, 30.0 and 100.0 mg/kg-day. In order to achieve steady state concentrations, the models were run until the predicted peak and trough heights did not change from one day to the next. Serum and thyroid AUCs were then determined over a 24 hr period (240-264 hrs in male, lactating and neonatal rats; 480-504 hrs in pregnant and fetal rats). Although the tissues reach steady state perchlorate concentrations within one week, the above time-points were chosen in the lactation and gestation models for their ability to be verified with data (Clewell *et al.*, 2001a and b). The male rat model was run at the same time as lactation for the sake of consistency with the other models. The total AUC in the serum and thyroid were determined from each the models at 240 and 264 hrs (or 480 and 504 hrs). The difference in the two values was then divided by 24 hrs to give the AUC in units of ng/L-hr.

Up-regulation of Thyroid ClO_4^- Uptake

During rat ClO_4^- drinking water studies, thyroidal iodide inhibition initially causes a decrease in T_4 and T_3, which initiates the positive feedback loop of the thyroid hormone system (Wolff, 1998). The rat hypothalamus-pituitary-thyroid axis quickly compensates for this inhibition of iodide uptake by increasing the amount of NIS, thereby increasing thyroidal iodide uptake to normal levels. Experiments (Yu *et al.*, 2000a and b) have shown this up-regulation to be both time and dose-dependent. Thus, at lower doses, the rat thyroid was completely up-regulated after only a few days of drinking water exposure. Iodide uptake in the thyroid at higher ClO_4^- doses ($\geq$10 mg/kg-day) was completely restored by the 18th day of exposure, which was the time of data collection in the pregnant and fetal rats (Clewell *et al.*, 2001b).

Drinking water studies in male rats showed elevated ClO_4^- uptake in the thyroid at drinking water doses of 3.0 mg/kg-day and higher (Yu *et al.*, 2000a; Merrill *et al.*, 2001a). Increased ClO_4^- uptake also results from up-regulation of NIS. Since perchlorate is transferred into the thyroid via NIS, the inhibiting anion is "up-regulated" along with iodide. In order to simulate increased ClO_4^- concentrations in thyroids of the 3.0, 10.0 and 30.0 mg/kg-day dose groups, the original value for follicular Vmax (Vmaxc_TP) was adjusted to obtain the best fit of the model simulation to experimental data (Table 1). Since there were no pharmacokinetic data available for the 5.0 and 100.0 mg/kg-day dose groups, values for Vmaxc_TP were estimated from a Michaelis-Menten (M-M) fit to the adjusted Vmax's at 3.0, 10.0 and 30.0 mg/kg-day doses (Figure 1). Target dosimetrics in the male rat were calculated for both originally optimized parameters and these "up-regulated" parameters. This process was not necessary in the gestation, lactation or human models, as they were able to successfully describe perchlorate concentrations in serum and thyroid at all measured doses (0.01 – 10.0 mg/kg-day in gestation and lactation, 0.02 – 12 mg/kg-day in human), using one set of model parameters (Clewell *et al.*, 2001a and b; Merrill *et al.*, 2000b).

Increased follicular Vmax values were not needed to fit the human data due partly to the larger size of the human thyroid colloid versus that of the rat. The human colloid has a greater capacity to reserve iodide than the rat. Humans also have thyroxine-binding globulin (TBG), which is not present in adult rats. TBG has a binding affinity to T_4 that is approximately 1000 times higher

than the binding affinity of prealbumin (the predominant plasma binding protein in rats), resulting in a biological half-life for T_4 of 5 to 9 days in humans versus 12 to 24 hrs in rats (Dohler *et al.*, 1979). As a result, iodide deficient humans can maintain normal hormone levels much longer before signaling for up-regulation by the hypothalamus-pituitary-thyroid axis.

In pregnant and lactating rats, it is likely that loss of maternal iodide to the fetus and neonate causes dams to exist in a chronic state of thyroidal up-regulation. As a result, the effect of perchlorate on the thyroid is less dramatic than in the male rat, where a completely naïve system is perturbed by an inhibiting chemical. Thus, the PBPK models for gestation and lactation were able to describe thyroid ClO_4^- levels at drinking water doses from 0.01 to 10.0 mg/kg-day without adjusting the follicular Vmax values with dose.

Table 1. "Up-regulated" Values of Vmaxc_TP after Drinking Water ClO_4^- Exposure in the Male Rat

Drinking Water Dose (mg/kg-day)	Adjusted Vmaxc_TP (ng/hr-kg)
0.01	2900
0.1	2900
1	2900
3	9000
5	17500*
10	32000
30	55000
100	79000*

* Data not available for these dose levels

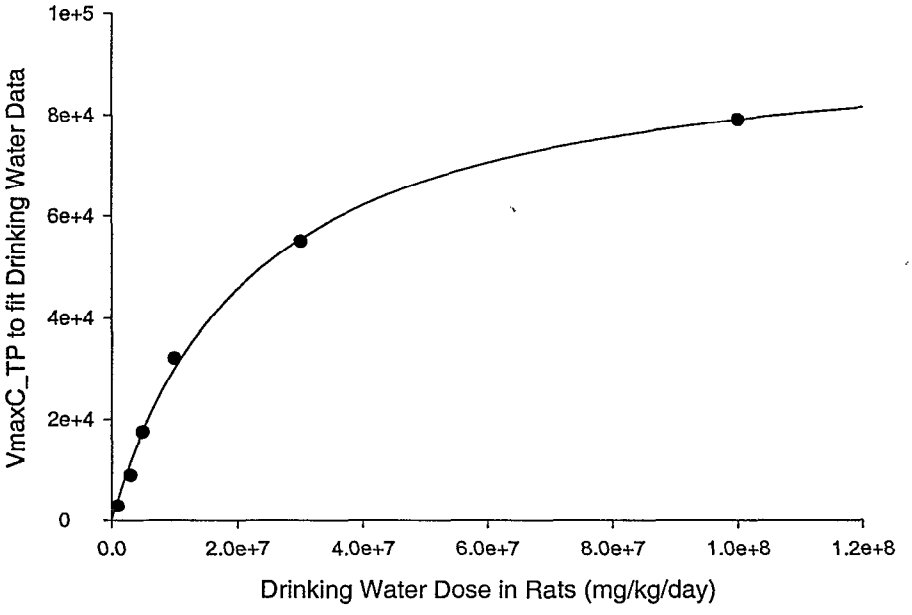


Figure 1. Up-regulation of Vmax_TP for ClO_4^- transport into thyroid follicle. Up-regulation is first noted in the 3.0 mg/kg-day dose group.

Dosimetrics in Human Model

The human PBPK model (Merrill *et al.*, 2001b) was used to calculate all target dosimetrics in both acute and two-week drinking water ClO_4^- exposures in a 70 kg adult at doses of 0.01, 0.1, 1.0, 3.0, 5.0, 10.0, 30.0 and 100.0 mg/kg-day. Acute serum and thyroid AUC values were calculated with the model over an eight hr time period (from 24 to 32 hrs post-exposure), in order to correlate ClO_4^- parameters to data-validated iodide inhibition. For two-week drinking water exposures, the thyroid and serum AUCs were calculated over a 24 hr period after serum and thyroid concentrations reached steady state. The 240 to 264 hr time period was chosen for consistency with the male rat model (Merrill *et al.*, 2001a). Actual human iodide inhibition data (Greer *et al.*, 2000) were plotted as a function of the ClO_4^- serum and thyroid dosimetrics calculated with the model (Figures 5 and 6).

The adult human model was also used to predict dosimetry in a 15 kg child. The same dosimetrics were run in the child and adult. However, since an average child drinks less water than an adult (approximately 1 L as opposed to 2 L in the adult), the actual exposures of a child and adult from the same water source would be different. For example, a 15 kg child consuming 1 L of contaminated water would receive a daily dose (per kg bodyweight) that was 2.3 times that of a 70 kg adult consuming 2 L of water. Table 2 shows the concentration of the drinking water required to deliver the same dose to a 15 kg child and a 70 kg adult. For the purpose of this paper, dosimetric comparisons were calculated using the same dose (mg/kg-day) in the adult and child. However, drinking water values were also determined assuming the same drinking water concentration (ng/L) (see Table 10 of Attachment 2).

Table 2. Drinking Water (DW) ClO_4^- Concentration Required to Deliver the Same Dose in a 15 kg Child and a 70 kg Adult

DW dose (mg/kg-day)	DW concentration (ng/L) 70 kg adult at 2 L/day	DW concentration (ng/L) 15 kg child at 1 L/day
0.01	350	150
0.1	3,500	1,500
1	35,000	15,000
3	105,000	45,000
5	175,000	75,000
10	350,000	150,000
30	1,050,000	450,000
100	3,500,000	1,500,000

Human Equivalent Exposure Dose

Human equivalent exposure doses (HEED) were calculated with the PBPK models, based on various internal dosimetrics. The HEED is estimated from human model simulations, which result in equivalent internal doses (e.g., AUC serum ClO_4^- or percent inhibition of iodide uptake) to those achieved in the rat under experimental dosing regimen (e.g., 0.1 mg/kg). The PBPK models are designed to account for species differences in kinetics of chemicals in the body. In the case of perchlorate, several species differences exist that affect perchlorate and inhibition kinetics. In addition to differences such as lower serum binding of perchlorate and greater thyroidal iodide uptake, humans also appear to have a greater capacity for reserving thyroid hormones than the rat (Dohler *et al.*, 1979). As a result, the human subjects (both sexes, from ages 22 to 57) did not exhibit thyroidal up-regulation within the two-week study period (Merrill *et al.*, 2001b; Greer *et al.*, 2000) as did rats. Neither thyroid radioiodide uptake measurements nor serum TSH levels indicated that perchlorate reduced iodide uptake or hormone synthesis enough to induce up-regulation of the NIS (Merrill *et al.*, 2001b). On the other hand, the rat is quickly forced into a hypothyroid state by ClO_4^- exposure and the system appears to be fully up-regulated within two weeks (Yu *et al.*, 2000a and b). Therefore, it was necessary to compare human drinking water exposures with acute *iv* exposures (naïve thyroid) in the rat. The following method was employed to calculate the HEED, correlating sub-chronic drinking water perchlorate levels in serum and thyroid of the male rat and human to the percent inhibition of iodide uptake in the naïve rat.

Decrements in percent inhibition only occurred in the male rat after acute *iv* dosing. The rat drinking water studies showed no iodide inhibition due to the rapid compensatory mechanism of the hypothalamus-pituitary-thyroid axis (Yu *et al.*, 2000a). Thus, the relationship between the blood and thyroid levels and the percent inhibition of iodide uptake in the thyroid was first established by plotting the experimentally determined percent inhibition against the PBPK model derived AUC for ClO_4^- in the serum and thyroid of the naïve rat (see Figures 2 and 3). A description of the resulting curve was then given by a Michealis-Menten (M-M) type equation.

In order to relate drinking water perchlorate levels to acute inhibition, model derived AUC values for sub-chronic exposures were input into the M-M equation to calculate the theoretical percent inhibition based on blood and thyroid levels when up-regulation has not been induced. Thus, serum and thyroid perchlorate levels in chronically exposed rats were correlated to thyroid iodide inhibition in naïve rat thyroids.

These calculations used AUC values, as opposed to peak concentrations, under the assumption that these dosimetrics would represent an averaging of serum and thyroid ClO_4^- concentration and would be better correlated with the inhibition effect. Peak ClO_4^- serum and thyroid concentrations after *iv* dosing are experimentally more variable, due to the rapid phase of distribution. Using simulated peak concentrations after *iv* injections is potentially problematic due to the inexact modeling of the actual distribution of dose in the tail vein volume and the exact time of mixing in the whole blood compartment. These two factors contribute uncertainty to model-simulated peak concentrations as an internal dosimetric.

In the human, up-regulation was not yet induced after two weeks of ClO_4^- exposure via drinking water. Therefore, the serum and thyroid levels from sub-chronic exposure were easily compared to the inhibition of iodide uptake in the naïve thyroid. As in the rat, a relationship between the serum and thyroid ClO_4^- levels and thyroidal inhibition were established by fitting a M-M equation to a plot of experimentally determined percent inhibition values versus the serum or thyroid ClO_4^- AUC concentrations at exposure day 2. Assuming that an equivalent human effect would constitute the same amount of inhibition in the thyroid, calculated values for percent inhibition in the rat are input into the human M-M equation relating thyroid inhibition to the AUC in either the serum or thyroid. The human model is then run to determine the external dose that would yield this calculated AUC. The resulting external dose is the HEED, or the external dose to humans associated with some effect in the rat. For the purpose of this report, HEEDs were determined for both 15 and 70 kg humans at all doses in which dosimetrics were calculated for the rat (0.01, 0.1, 1.0, 3.0, 5.0, 10.0, 30.0 and 100.0 mg/kg-day).

Sensitivity Analysis

A sensitivity analysis was performed on the male rat model in order to determine which parameters had the most significant impact on serum and thyroid AUCs. All chemical specific kinetic parameters were increased individually by 1% from the original, optimized values. The model predicted dosimetrics were re-calculated after each change to determine the effect on the AUCs. This exercise was performed at the 0.1 and 1.0 mg/kg-day doses. The equation describing the calculation of the Sensitivity Coefficient value for each PBPK perchlorate parameter tested is:

$$\text{Sensitivity Coefficient} = \frac{(A - B)/B}{(C - D)/D}$$

Where A = AUC for either serum or thyroid with 1% increased parameter value
B = AUC for either serum or thyroid at starting parameter value
C = Parameter value increase 1% over starting parameter value
D = Original starting parameter value

RESULTS AND DISCUSSION

Dose-Response Relationships

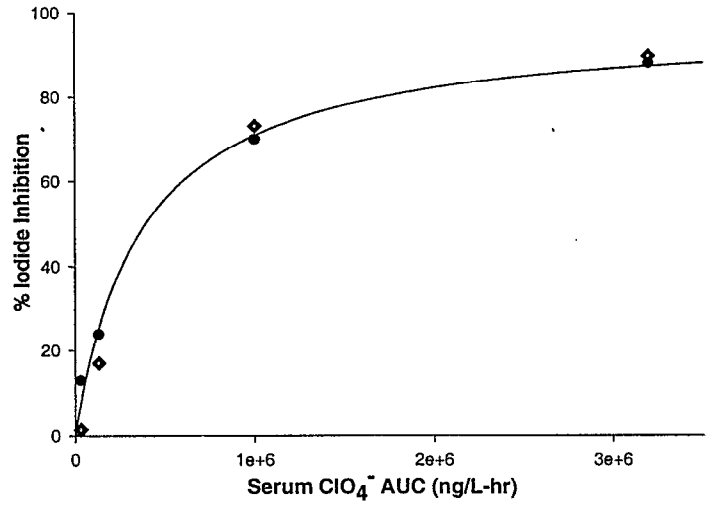
Figure 2 shows the curve generated from plotting the experimentally determined percent inhibition versus the corresponding PBPK-derived serum AUC after acute ClO_4^- exposure in rats. Thyroidal $^{125}I^-$ uptake measurements were taken two hours after *iv* administration of perchlorate. The solid line represents a fit (not a PBPK model simulation) using the Michaelis-Menten (M-M) type equation given below:

$$Y = (A \times AUC_{dose})/(AUC_{dose} + B)$$

Where 'Y' represents the predicted percent inhibition of radioiodide uptake, 'A' represents the maximal percent inhibition of radioiodide uptake, 'B' is related to the affinity of iodide uptake based on serum concentration, and AUC_{dose} represents the AUC at each specific dose of perchlorate. The above equation was also used to derive the dose-response curves in Figures 3 through 8. Table 3 lists the parameters used for each figure and the correlation coefficient or goodness of fit (r^2).

Table 3. Michaelis-Menten Equation Parameters

Figure	Equation Parameter A (max % inhibition)	Equation Parameter B (ng/L)	Correlation Coefficient (r^2)
2	100	408,622	0.9910
3	100	5,865,313	0.9079
4	100	408,204	0.9986
5	100	5,854,650	0.9999
6	100	163,839	0.9528
7	100	107,591,935	0.9896
8	100	3,211,874	0.9999
9	100	38,337,137	0.9999



• - represents experimental data
♦ - represents PBPK model simulation values

Figure 2. Michaelis-Menten fit of the acute male rat AUC for serum ClO_4^- (PBPK-derived) after *iv* injection vs. experimentally determined percent inhibition of radioiodide uptake

Measured percent inhibition of radioiodide uptake in the thyroid after acute ClO_4^- exposure in male rats versus model-predicted AUC of thyroidal perchlorate from 2 to 4 hours post-dosing after *iv* administration of perchlorate in male rats is presented in Figure 3. The solid line represents a fit (not a PBPK model simulation) using the Michaelis-Menten nonlinear equation described above. The values for the M-M equation are given in Table 3.

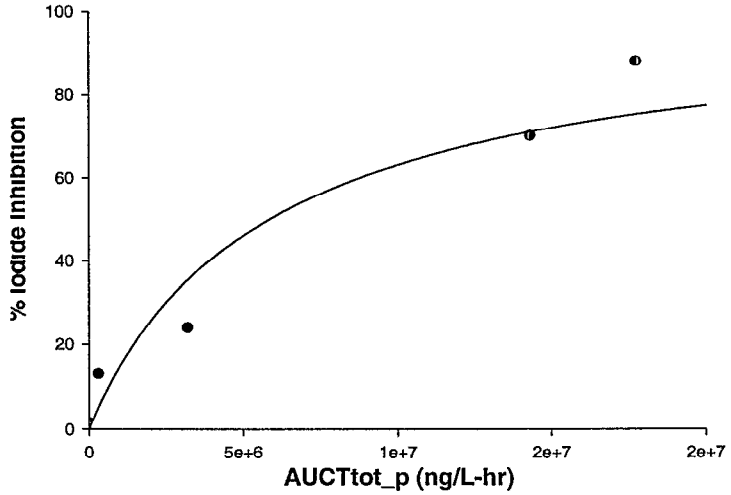


Figure 3. Michaelis-Menten fit of acute male rat AUC for thyroid ClO_4^- (PBPK-derived) after *iv* injection vs. experimentally determined percent inhibition of radioiodide uptake

Figure 4 shows the PBPK-derived AUC for serum ClO_4^- from drinking water exposure to the male rat vs. the calculated percent inhibition of radioiodide. The values for AUC of ClO_4^- in the serum were determined by running the male rat model (Merrill *et al.*, 2001a) across doses. Corresponding percent inhibitions were calculated by putting serum AUC values into the equation from Figure 2. Human response (thyroid inhibition) to sub-chronic exposure is similar to that of an acute exposure in the rat. This approach allows the sub-chronic serum levels in the rat to be related to iodide uptake in the naive thyroid.

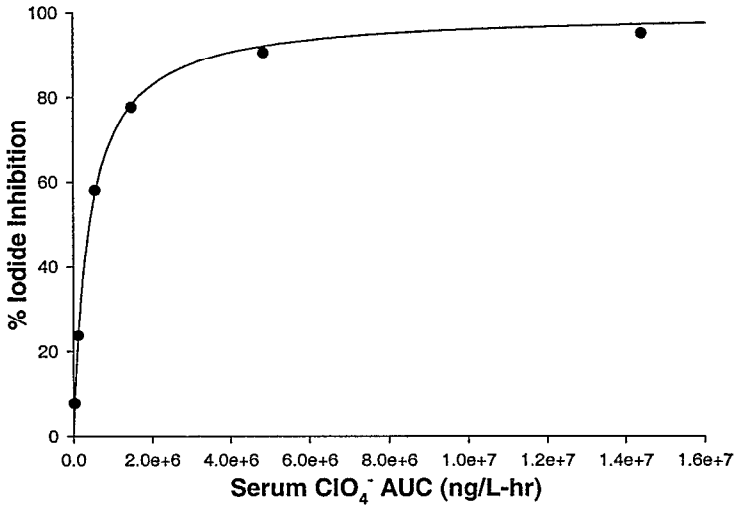


Figure 4. Michaelis-Menten fit of the male rat serum AUC from DW ClO_4^- (PBPK-derived) vs. percent inhibition of radioiodide uptake calculated from equation in Figure 2

Figure 5 is similar to Figure 4, except the percent inhibition of radioiodide uptake is calculated from the PBPK-derived AUC for thyroid ClO_4^-. The values for AUC of thyroid ClO_4^- concentration in Figure 5 were determined by running the male rat model (Merrill *et al.*, 2001a) at steady state (between 240 and 264 hours of drinking water exposure) across the doses shown. Corresponding percent inhibitions were calculated by putting thyroid AUC values into the equation from Figure 3.

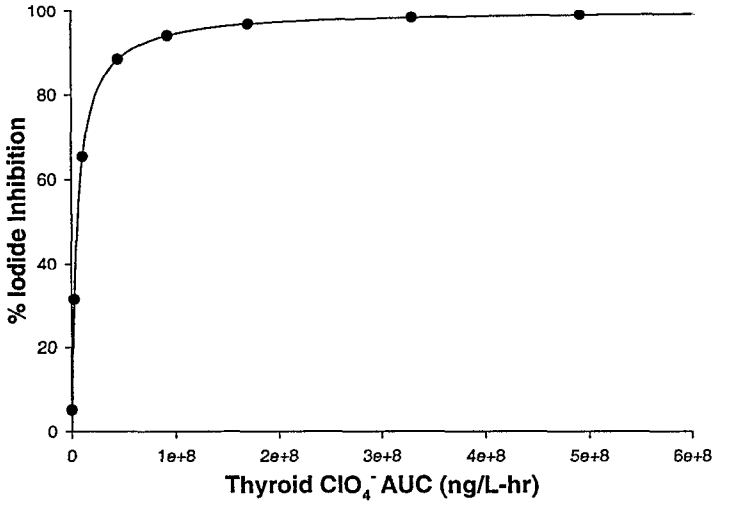


Figure 5. Michaelis-Menten fit of the PBPK-derived male rat AUC thyroid ClO_4^- from DW vs. percent inhibition of radioiodide uptake calculated from equation in Figure 3

Figure 6 presents the measured percent inhibition of radiolabeled iodide uptake in the thyroid on day 2 of drinking water exposure to perchlorate verses the PBPK-derived serum AUC for perchlorate in human volunteers (both male and female). Inhibition data from time points earlier than day 2 of perchlorate in the human drinking water study (Greer *et al.*, 2000) and inhibition data from acute perchlorate dosing in humans were not available. Therefore, the inhibition measurements on day 2 of perchlorate drinking water exposure were the closest available representation of an acute human dose. Measured serum TSH and thyroid hormones indicated that thyroids were in normal homeostatic state in human volunteers during the entire two week study (Merrill *et al.*, 2000b).

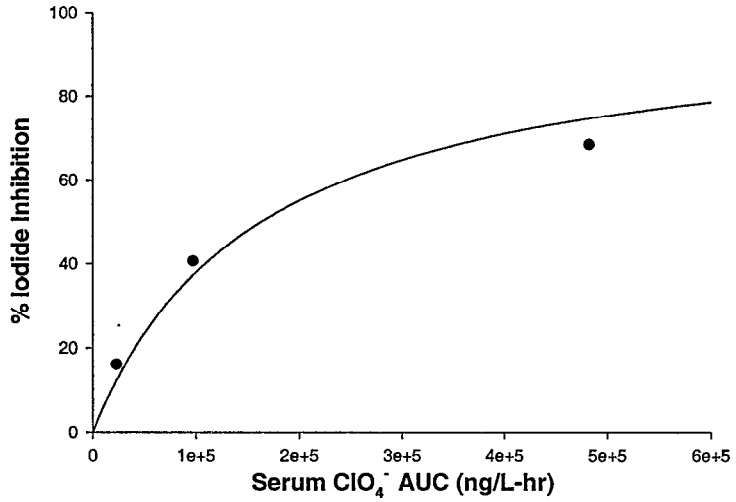


Figure 6. Michaelis-Menten fit of the human AUC for serum ClO_4^- on exposure day 2 (PBPK-derived) vs. calculated percent inhibition of radioiodide uptake

The PBPK-derived AUC for thyroid ClO_4^- in humans on the second day of drinking water exposure vs. percent inhibition of experimentally determined radioiodide is presented in Figure 7.

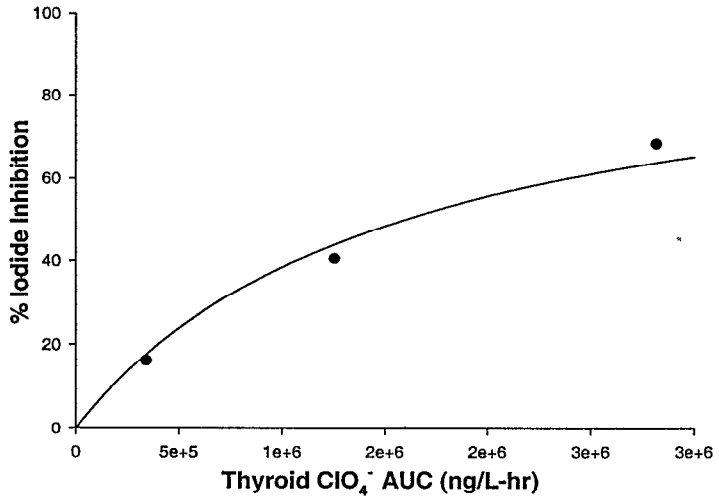


Figure 7. Michaelis-Menten fit of human AUC for thyroid ClO_4^- on exposure day 2 (PBPK-derived) vs. calculated percent inhibition radioiodide uptake

The HEED that would result in the same serum AUC for ClO_4^- in the human and rat; the subsequent percent inhibition of iodide uptake is presented in Figure 8. Values for percent inhibition were determined from the rat serum AUC during drinking water exposures to perchlorate using the M-M equation from Figure 2. Similarly, Figure 9 shows the HEED that would result in the same thyroid AUC(s) for ClO_4^- in the human and rat vs. the subsequent percent inhibition, using the equation calculated from Figure 3.

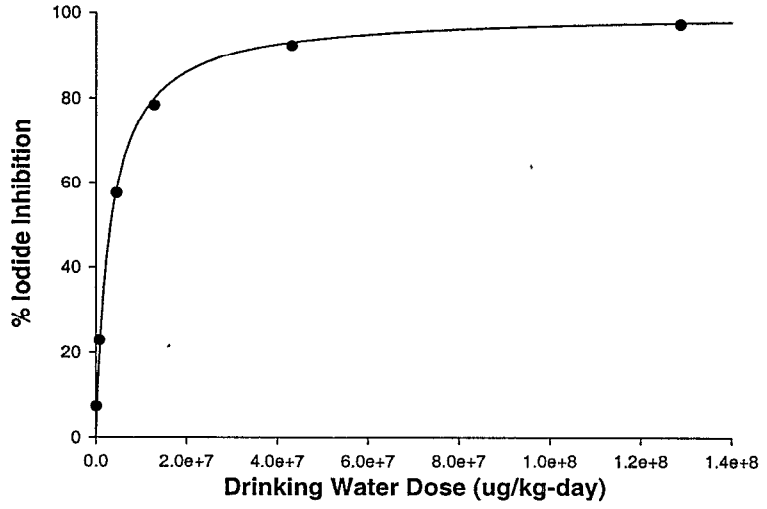


Figure 8. Michaelis-Menten fit of the HEED of ClO_4^- in drinking water derived from serum AUC vs. predicted acute percent inhibition in the rat (determined from Figure 2)

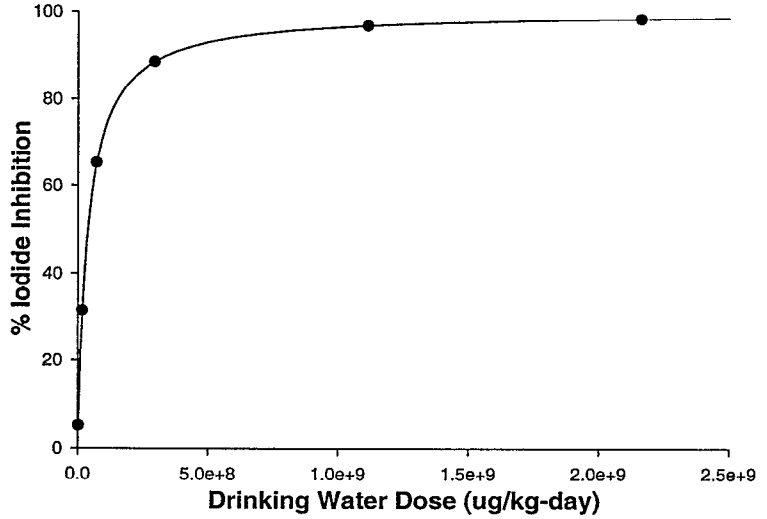


Figure 9. Michaelis-Menten fit of the HEED of ClO_4^- in drinking water derived from thyroid AUC vs. predicted acute percent inhibition in the rat (determined from Figure 3)

Internal Dosimetrics

In order to allow comparison of the four models, the same parameters (serum and thyroid AUC, serum and thyroid peak concentrations, urinary excretion and percent inhibition) were analyzed in drinking water and acute *iv* exposure scenarios in all of the models. Internal dosimetrics for male, pregnant, lactating, fetal and neonatal rats, as well as adult and 15 kg humans are presented in Attachment 2. The AUC for serum and thyroid ClO_4^- concentrations, peak serum and thyroid ClO_4^- concentrations, total amount of ClO_4^- excreted in the urine and percent inhibition of radioiodide uptake into the thyroid were calculated (Attachment 2, Tables 1 through 11). In addition, AUCs for lactational and placental transfer of ClO_4^- (Table 12 of Attachment 2) were calculated using the lactation and pregnancy models by Clewell *et al.* (2001a and b). The dosimetrics in Attachment 2 are provided by dose level (both from acute and drinking water exposures).

In real world scenarios, however, adults and children receive different doses from drinking water consumption due to differences in bodyweight and the amount of water consumed (2 L/day vs 1 L/day). To address the issue of internal dosimetrics from the same external exposure for both adults and children, drinking water dosimetrics were calculated for the child and adult receiving the same water concentration (Table 4). Based on drinking water concentration, the dose to the child is approximately twice that received by the adult.

Table 4. Comparison of PBPK-Derived Dosimetrics in 15 kg Child and 70 kg Adult Consuming the Same Perchlorate Concentration

Adult DW Dose (mg/kg-day)	Corresponding Child DW Dose (mg/kg-day)	Child DW AUC Thyroid (ng/L-hr)	Adult DW AUC Thyroid (ng/L-hr)	Child DW AUC Serum (ng/L-hr)	Adult DW AUC Serum (ng/L-hr)	Child 24-hr urine (ng/24 hr)	Adult 24-hr urine (ng/24 hr)
0.01	0.023	2.77E+07	1.83E+07	3.14E+04	1.78E+04	3.45E+05	7.00E+05
0.1	0.23	1.50E+08	1.18E+08	1.92E+05	1.22E+05	3.45E+06	7.00E+06
1.0	2.3	2.78E+08	2.65E+08	1.67E+06	1.07E+06	3.45E+07	7.00E+07
3.0	7.0	3.02E+08	2.95E+08	5.01E+06	3.16E+06	1.05E+08	2.10E+08
5.0	12	3.10E+08	3.04E+08	8.36E+06	5.25E+06	1.76E+08	3.50E+08
10	23	3.20E+08	3.14E+08	1.66E+07	1.05E+07	3.50E+08	7.00E+08
30	70	3.52E+08	3.35E+08	4.98E+07	3.14E+07	1.05E+09	2.10E+09
100	233	4.52E+08	4.00E+08	1.66E+08	1.05E+08	3.50E+09	7.00E+09

Adult DW Dose (mg/kg-day)	Corresponding Child DW Dose (mg/kg-day)	Child Peak Serum (ng/L)	Adult Peak Serum (ng/L)	Child Peak Thyroid (ng/L)	Adult Peak Thyroid (ng/L)
0.01	0.023	8.96E+05	2.13E+04	2.43E+08	1.95E+07
0.1	0.23	8.96E+05	1.59E+05	2.43E+08	1.27E+08
1.0	2.3	2.75E+06	1.54E+06	2.89E+08	2.73E+08
3.0	7.0	8.32E+06	4.60E+06	3.08E+08	2.99E+08
5.0	12	1.39E+07	7.66E+06	3.15E+08	3.07E+08
10	23	2.76E+07	1.53E+07	3.28E+08	3.17E+08
30	70	8.30E+07	4.59E+07	3.70E+08	3.43E+08
100	233	2.76E+08	1.53E+08	5.11E+08	4.23E+08

Note: Corresponding child dose is based on consumption of 1 L/day vs. 2 L/day for adult

Comparison of Dosimetric Ratios

Ratios of the internal dosimetrics between models are presented in Tables 5 through 9b. The rat serum ratios (AUC and peak concentrations) change significantly between 0.1 and 3.0 mg/kg-day, due to binding of ClO_4^- by plasma proteins (Tables 5 and 6). Plasma binding is saturated at doses greater than 1.0 mg/kg-day. Male rat to human serum ratios are notably lower than those ratios between rats, as plasma binding of perchlorate occurs to a much lesser extent in humans.

Table 5. Ratios of PBPK-derived AUC Serum Concentrations from Drinking Water

Dose (mg/kg-day)	Male Rat: Human	Male Rat: Pregnant Rat	Male Rat: Lactating Rat	Male Rat: Fetal Rat	Male Rat: Neonate Rat	Pregnant Rat: Fetal Rat	Lactating Rat: Neonate Rat
0.01	1.81	0.63	0.58	1.44	1.16	2.28	1.99
0.1	0.99	0.73	0.54	1.06	0.85	1.46	1.56
1.0	0.51	0.90	0.84	1.44	1.01	1.61	1.20
3.0	0.46	0.94	0.95	1.67	1.71	1.77	1.80
5.0	0.45	0.95	0.98	1.74	2.14	1.82	2.18
10.0	0.44	0.96	1.01	1.80	2.70	1.87	2.69
30.0	0.44	0.97	1.02	1.84	3.33	1.90	3.26
100.0	0.44	0.97	1.03	1.85	3.65	1.92	3.55

Table 6. Ratios of PBPK-derived Peak Serum Concentrations from Drinking Water

Dose (mg/kg-day)	Male Rat: Human	Male Rat: Pregnant Rat	Male Rat: Lactating Rat	Male Rat: Fetal Rat	Male Rat: Neonate Rat	Pregnant Rat: Fetal Rat	Lactating Rat: Neonate Rat
0.01	1.85	0.67	5.29	1.47	1.38	2.18	0.26
0.1	0.92	0.76	0.61	1.09	1.00	1.43	1.66
1.0	0.57	0.93	1.00	1.59	1.60	1.72	1.60
3.0	0.55	0.97	1.11	1.81	2.92	1.86	2.63
5.0	0.55	0.98	1.14	1.86	3.69	1.90	3.24
10.0	0.55	1.01	1.19	1.94	3.40	1.93	2.86
30.0	0.55	1.01	1.21	1.98	5.96	1.95	4.95
100.0	0.55	1.01	1.21	1.99	6.53	1.96	5.39

In determining ratios of thyroid AUCs (Tables 7 and 8), it was necessary to use the up-regulated values for thyroid uptake (Vmaxc_TP) in the male rat. Without the adjusted Vmaxc_TP, the male rat model was not able predict the thyroid ClO_4^- concentrations at drinking water doses of 3.0 mg/kg-day and higher. It should be noted that ratios from doses higher than 30 mg/kg-day are hypothetical estimates, as no data at that dose level were available to verify the male rat simulations. The adjusted values for Vmaxc_TP, required to simulate the AUC's of thyroid ClO_4^- from those doses, were estimated from the equation generated from the data in the 0.01, 0.1, 1.0, 3.0 and 10.0 mg/kg-day doses in Figure 1.

Uptake parameters were not adjusted in the human, pregnant rat or lactating rat models. Hence, a notable change in ratios of AUC and peak thyroid concentrations between the male rat and other subjects occur at 3.0 mg/kg-day and higher. The rapid up-regulation did not occur in humans within the two week study period (Greer *et al.*, 2000; Merrill *et al.*, 2001b). In pregnant and lactating rats, rapid up-regulation was evidenced by increased serum TSH levels during exposure (Yu *et al.*, 2000b; Clewell *et al.*, 2001a and b). However, due to increased transfer of perchlorate to the fetus and suckling neonate, as well as changing tissues volumes, thyroid

concentrations in pregnant and lactating rats did not reach concentrations seen in male rat thyroids.

It is also important to recognize that in a few cases, it was not possible to verify model predictions against experimental data. For example, fetal and neonatal rat and human thyroids, were never actually analyzed for perchlorate concentration. In the adult human, perchlorate thyroid kinetics were assumed to be similar to those for iodide. In the case of the fetus, kinetic parameters were determined by fitting the model simulation of fetal thyroid concentrations to available iodide data and assuming that the perchlorate:iodide ratio would be similar to the mother's. In the case of the neonatal rat, no data were available for thyroid concentrations in either perchlorate or iodide. Thus, model predictions are based on allometrically scaled maternal parameters for thyroid uptake. Therefore, values presented in Tables 7 and 8 (and Tables 2 and 4 of Attachment 2) for the perchlorate concentration in human and fetal and neonatal thyroids are purely predictive, based on iodide data available.

It is the opinion of the authors that, while the thyroid perchlorate parameters in the human and rat fetus and neonate are highly informative, they should not be used in a formal risk assessment, especially in the case of the neonatal thyroid. However, many of the parameters in the models, such as maternal serum, thyroid and milk, fetal/pup serum and total body burden, are well verified with both perchlorate and iodide data at different developmental time-points and various doses (Clewell *et al.*, 2001a and b). Also, the models successfully describe both drinking water distribution and acute kinetics of perchlorate and iodide in the mother, fetus and neonate. Therefore, they still provide a useful tool for predicting previously unknown parameters, the neonatal and fetal dose and serum concentrations (Clewell *et al.*, 2001a and b).

Table 7. Ratios of PBPK-derived AUC Thyroid ClO_4^- Concentrations from Drinking Water

Dose (mg/kg-day)	Male Rat: Human	Male Rat: Pregnant Rat	Male Rat: Lactating Rat	Male Rat: Fetal Rat[b]	Male Rat: Neonate Rat	Pregnant Rat: Fetal Rat	Lactating Rat: Neonate Rat
0.01	0.02	0.26	0.68	0.23	1.52	0.86	2.24
0.1	0.02	0.31	0.64	0.15	1.08	0.49	1.70
1.0	0.04	0.45	0.64	0.26	1.61	0.59	2.51
3.0	0.19	1.64	2.26	1.22	6.89	0.74	3.05
5.0	0.38	2.73	3.71	2.06	12.53	0.75	3.38
10.0	0.72	3.61	4.79	3.51	18.97	0.97	3.96
30.0	1.22	2.91	3.74	4.64	18.80	1.59	5.02
100.0	1.52	1.48	1.87	3.68	11.02	2.49	5.89

Notes: Used up-regulated Vmaxc_Tp for perchlorate in the male rat thyroid follicle (see Table 1)
Thyroid concentration data for fetal and neonate rat were not available to confirm AUC for thyroid ClO_4^-

Table 8. Ratios of PBPK-derived Peak Thyroid Concentrations from Drinking Water

Dose (mg/kg-day)	Male Rat*: Human	Male Rat: Pregnant Rat	Male Rat: Lactating Rat	Male Rat: Fetal Rat	Male Rat: Neonate Rat	Pregnant Rat: Fetal Rat	Lactating Rat: Neonate Rat
0.01	0.02	0.30	8.23	0.25	2.03	0.83	0.25
0.1	0.03	0.36	0.77	0.26	1.51	0.71	1.96
1.0	0.05	0.48	0.71	0.28	2.08	0.58	2.92
3.0	0.17	1.18	1.69	0.85	6.26	0.73	3.69
5.0	0.28	1.50	2.14	1.32	9.13	0.88	4.26
10.0	0.58	2.02	2.87	2.45	12.14	1.21	4.22
30.0	1.06	1.61	2.28	3.22	16.27	2.00	7.13
100.0	1.70	1.04	1.47	2.98	12.65	2.87	8.61

Notes: Used up-regulated Vmaxc_Tp for perchlorate in the male rat thyroid follicle (see Table 1)
Thyroid concentration data for fetal and neonate rat were not available to confirmed AUC thyroid ClO_4^-

Table 9. Ratios of (PBPK-derived) Percent DW Dose Eliminated in 24-hr Urine

Dose (mg/kg-day)	Male Rat: Human	Male Rat: Pregnant Rat	Male Rat: Lactating Rat	Male Rat: Fetal Rat	Male Rat: Neonate Rat	Pregnant Rat: Fetal Rat	Lactating Rat: Neonate Rat
0.01	0.94	0.97	1.85	NA	5.74	NA	3.31
0.1	0.94	0.97	1.64	NA	2.98	NA	1.94
1.0	0.94	0.96	1.17	NA	1.65	NA	1.50
3.0	0.94	0.96	1.09	NA	1.54	NA	1.51
5.0	0.94	0.96	1.07	NA	1.50	NA	1.50
10.0	0.94	0.96	1.06	NA	1.45	NA	1.47
30.0	0.94	0.96	1.05	NA	1.41	NA	1.44
100.0	0.94	0.96	1.04	NA	1.39	NA	1.42

Notes: NA - urinary output not applicable for fetus
No urine data available to verify values in the neonate

Table 9b. Ratios of (PBPK-Derived) % Inhibition of Thyroid Iodide Uptake after *iv* in rats and 2 wks DW in Humans Water

Dose Rats(mg/kg) Human(mg/kg/d)	Male Rat: 70 kg Human [a]	Male Rat: Pregnant Rat	Male Rat: Lactating Rat	Male Rat: Fetal Rat [b]	Male Rat: Neonate Rat [b]	Pregnant Rat: Fetal Rat [b]	Lactating Rat: Neonate Rat [b]
0.01	0.550	0.48	3.24	-0.01	4.02	-0.02	1.2
0.1	0.688	0.54	3.06	0.59	12.75	1.08	4.2
1	0.928	0.84	1.18	0.92	24.53	1.09	20.7
3	0.975	0.96	0.97	1.00	27.49	1.04	28.4
5	0.983	0.96	0.98	1.03	30.45	1.07	31.2
10	0.988	0.98	0.99	0.98	25.61	1.00	26.0
30	0.992	0.99	1.00	0.99	16.06	1.00	16.1
100	0.993	1.00	1.00	1.00	7.38	1.00	7.4

Notes: [a] Inhibition in human was PBPK-derived from 2 wks CLO_4^- DW exposure - all rat values simulated from *iv* dose.
[b] Model predicted inhibition in fetal and neonate rats not validated with data

From the tables presented above and in Attachment 2, it is apparent that the pregnant and lactating rats have significantly higher average serum concentrations in the lowest drinking water dose (0.01 mg/kg-day). This is likely due to increased binding in the serum. It has been shown that the estrus cycle affects the concentration of binding proteins within the blood. Thyroxine, which is displaced from plasma proteins by perchlorate, is bound to a greater extent in the pregnant rat (Iino and Greer, 1961). It follows, then, that perchlorate would also be bound to a greater extent during pregnancy and possibly lactation. Since serum binding affects only the low doses, it is reasonable that the higher doses (1.0 through 100.0 mg/kg-day) would be similar in male, pregnant and lactating female rats.

Both data and model simulations show increased thyroidal uptake of iodide in the pregnant rat as opposed to the male rat at low doses (below 3.0 mg/kg-day). This same behavior has been recorded by literature sources and has previously been attributed to a diminished reserve of bound iodide within the maternal thyroid. It is reasonable to assume that the iodide reserve is depleted in the pregnant rat, due to changes in hormone and iodide distribution, as well as loss to the fetus and urine. Additionally, changes in hormonal balance, especially estrogen, are known to affect thyroid hormone homeostasis. The pregnant rat evidently exists in an up-regulated state, where iodide is being actively stored and delivered to the developing fetus (Iino and Greer, 1961; Versloot *et al.*, 1997).

Although it would seem reasonable that the thyroid of the lactating rat would show the same increased uptake as the pregnant rat, both the data and model predictions indicate that iodide uptake is more similar to the male rat than the pregnant female (Clewell *et al.*, 2001a; Yu *et al.*, 2000b). This is not surprising when loss to maternal milk is taken into consideration. The pregnant and lactating rat models utilize identical parameters to describe data that differ by as much as an order of magnitude. This suggests that uptake kinetics are the same in the pregnant and lactating rat (Clewell *et al.*, 2001a and b). Therefore, differences in the physiology of the two reproductive stages must account for the difference in thyroidal iodide levels. The use of the model reveals that milk provides a significant source of excretion for maternal iodide. In fact, the high affinity of the mammary gland and milk may be the reason that the maternal thyroid is not able to achieve the high levels of perchlorate seen in the pregnant rat (Tables 7 and 8). Literature sources support this behavior, suggesting that the lactating mammary is more efficient at iodide uptake than the thyroid (Brown-Grant, 1961).

Human Equivalent Exposure Dose

In Tables 10 and 11, the calculated HEEDs are shown for 15 and 70 kg humans. The differences between the 15 and 70 kg human HEEDs were never greater than 75%, indicating that bodyweight doesn't significantly affect the target dosimetrics. Interestingly, the HEEDs were greater in the 15 kg child. One may expect the adult and child HEEDs to be nearly equal, given no parameters were changed in the human model except bodyweight. However, physiological parameters within the model are linearly scaled by bodyweight, whereas chemical-specific parmeters are scaled nonlinearly (e.g., as a multiple of bodyweight to a power of ¾). As indicated later in the sensitivity analysis, the internal dosimetrics presented are more sensitive to

to chemical-specific parameters, especially those describing saturable kinetics. Therefore, the chemical-specific parameter values for the 15 kg child are proportionally greater (in terms of bodyweight) than those of the adult. As a result, a slightly higher dose is required to saturate these tissues in a child.

It should be kept in mind that the HEEDs represent dose and not drinking water concentration. As shown earlier in Table 2, the 15 kg child would receive approximately 2.3 times the dose received by an adult drinking water containing the same perchlorate concentration (given the adult drinks 2 L/day and the child drinks 1 L/day).

When comparing the dosimetrics for serum versus thyroid, the HEED calculated from the thyroid was less than the HEED calculated from the serum by a factor of 100 at a 0.01 mg/kg-day dose level. This difference became a factor of 10 starting at the 5.0 mg/kg-day concentration for the 15 kg child and at 10.0 mg/kg-day for the adult.

Table 10. PBPK-derived 15 and 70 kg Human Equivalent Exposure Doses Based on PBPK-derived Serum and Thyroid AUC(s) in the Male Rat

Rat DW Concentration (mg/kg-day)	Human 15 kg HEED (mg/kg-day) based on male rat serum*	Human 70 kg HEED (mg/kg-day) based on male rat serum*	Human 15 kg HEED (mg/kg-day) based on male rat thyroid*	Human 70 kg HEED (mg/kg-day) based on male rat thyroid*
0.010	0.030	0.021	0.0002	0.0001
0.1	0.145	0.100	0.002	0.001
1.0	0.745	0.505	0.008	0.006
3.0	2.05	1.35	0.052	0.035
5.0	3.35	2.25	0.145	0.098
10.0	6.75	4.45	0.725	0.460
30.0	20.3	13.2	163	110
100.0	65.0	43.8	490	330

Note: * Calculated from PBPK-derived rat AUC(s) at steady state between 240 and 264 hrs during DW exposure, using up-regulated Vmaxv_TP values from Table 1

The HEEDs in Table 11 represent the equivalent drinking water doses to normal adults and children that would result in the same level of inhibition predicted in the rat from an acute *iv* dose of perchlorate. The inhibition response in humans after 2 weeks of drinking water exposure is very similar to that in the rat 2 hrs after *iv* doses up to 10.0 mg/kg. The HEEDs calculated using equivalent levels of iodide inhibition as seen in the male rat were closer to the actual rat doses than the HEEDs based on equivalent perchlorate concentrations in serum or thyroid. In addition, the HEED values in Table 11 are representative of the endpoint (thyroid iodide inhibition). Above 10 mg/kg, the human appears more sensitive to perchlorate-induced inhibition. It should be noted that validation data in the male rat were not available at the *iv* doses of 30.0 and 100.0 mg/kg.

Table 11. PBPK-derived 15 and 70 kg Human Equivalent Exposure Doses Based on PBPK-derived Thyroid Inhibition in the Male Rat

Dose -(mg/kg) for Rat -(mg/kg-day) for 15 and 70 kg Humans	PBPK-Derived % Inhibition in Rat 2 hrs after *iv* dose*	PBPK-derived 15 kg Human HEED (mg/kg-day) based on % inhibition seen in rat after *iv* dose	PBPK-derived 70 kg Human HEED (mg/kg-day) based on % inhibition seen in rat after *iv* dose
0.01	1.5%	0.006	0.004
0.1	16.3%	0.075	0.048
1.0	74.5%	1.5	0.9
3.0	90.0%	4.8	2.7
5.0	93.5%	8.0	4.9
10.0	96.2%	16.0	9.0
30.0	98.1%	35.0	19.3
100.0	98.7%	50.0	33.0

Sensitivity Analysis

An analysis of model parameter sensitivity on AUC serum and thyroid concentrations was performed with the male rat model (Merrill *et al.*, 2001a) using a 4 hr time-point after *iv* dosing of 0.1 and 1.0 mg/kg ClO_4^- (Tables 12 through 15). Each model parameter was increased one percent. The changes in predicted area under the curve for serum and thyroid concentrations are presented in the final two columns.

Table 12. Sensitivity Analysis for Physical Parameters in the Male Rat Model at 0.1 mg/kg ClO_4^-

Parameter	Original Parameter Value	1% Increase in Parameter Value	AUC Thyroid Sensitivity Coefficient	AUC Serum Sensitivity Coefficient	Increase in AUC Thyroid ClO_4^- (ng)	Increase in AUC Serum ClO_4^- (ng)
BW	3.00E-01	3.03E-01	0.315	0.182	1.88E+06	9.95E+04
Blood Flows [fraction of cardiac output, QCc (L/hr)]						
QCc	1.40E+01	1.41E+01	-0.005	-0.006	1.88E+06	9.94E+04
QTc	1.60E-02	1.62E-02	NS	NS	1.88E+06	9.94E+04
QSKc	5.80E-02	5.86E-02	NS	-0.003	1.88E+06	9.94E+04
QGc	1.60E-02	1.62E-02	0.011	0.008	1.88E+06	9.94E+04
QLc	1.70E-01	1.72E-01	NS	NS	1.88E+06	9.94E+04
QKc	1.40E-01	1.41E-01	-0.016	-0.010	1.88E+06	9.93E+04
QFc	6.90E-02	6.97E-02	NS	NS	1.88E+06	9.94E+04
Tissue Volumes (fraction of bodyweight)						
Vplasc	4.10E-02	4.14E-02	0.155	0.079	1.88E+06	9.94E+04
VRBCc	3.30E-02	3.33E-02	0.192	0.109	1.88E+06	9.95E+04
Vttotc	7.70E-05	7.78E-05	0.187	0.113	1.88E+06	9.95E+04
VDTc	2.44E-01	2.46E-01	0.928	0.114	1.89E+06	9.95E+04
VTBc	1.57E-01	1.58E-01	0.203	0.114	1.88E+06	9.95E+04
VTc	6.00E-01	6.05E-01	0.453	0.114	1.88E+06	9.95E+04
VGc	5.40E-03	5.45E-03	0.197	0.112	1.88E+06	9.95E+04
VGJc	1.68E-02	1.70E-02	0.165	0.091	1.88E+06	9.94E+04
VGBc	4.10E-02	4.14E-02	0.197	0.114	1.88E+06	9.95E+04
VSkc	1.90E-01	1.92E-01	-0.053	-0.023	1.87E+06	9.93E+04
VSkBc	2.00E-02	2.02E-02	0.203	0.117	1.88E+06	9.95E+04
VLc	5.50E-02	5.56E-02	0.197	0.114	1.88E+06	9.95E+04
VKc	1.70E-02	1.72E-02	0.197	0.113	1.88E+06	9.95E+04
VFc	7.40E-02	7.47E-02	0.208	0.118	1.88E+06	9.95E+04

Notes: NS = sensitivity coefficient less than 0.001
AUC Thyroid Concentration using original parameters = 1.88E+06 ng ClO_4^-
AUC Serum Concentration using original parameters = 9.94E+04 ng ClO_4^-

Table 13. Sensitivity Analysis for Chemical Specific Parameters in the Male Rat Model at 0.1 mg/kg ClO_4^-

Parameter	Original Parameter Value	1% Increase in Parameter Value	AUC Thyroid Sensitivity Coefficient	AUC Serum Sensitivity Coefficient	Increase in AUC Thyroid ClO_4^- (ng)	Increase in AUC Serum ClO_4^- (ng)
Iodide Tissue/Blood Partition Coefficients						
PS_p	3.10E-01	3.13E-01	0.149	0.085	1.88E+06	9.94E+04
PR_p	5.60E-01	5.66E-01	0.192	0.111	1.88E+06	9.95E+04
PK_P	9.90E-01	1.00E+00	0.192	0.111	1.88E+06	9.95E+04
PL_p	5.60E-01	5.66E-01	0.187	0.108	1.88E+06	9.95E+04
PG_p	1.80E+00	1.82E+00	0.160	0.088	1.88E+06	9.94E+04
PGJ_p	2.30E+00	2.32E+00	0.165	0.090	1.88E+06	9.94E+04
PT_p	1.30E-01	1.31E-01	1.184	0.113	1.90E+06	9.95E+04
PDT_p	7.00E+00	7.07E+00	0.928	0.114	1.89E+06	9.95E+04
PF_p	5.00E-02	5.05E-02	0.197	0.114	1.88E+06	9.95E+04
PSk_p	7.00E-01	7.07E-01	11.154	6.024	2.08E+06	1.05E+05
PRBC_p	8.00E-01	8.08E-01	11.324	6.112	2.09E+06	1.05E+05
Perchlorate Active Uptake Parameters - Vmaxc (ng/hr-kg BW) Km (ng/L)						
Vmaxc_Tp	2.90E+03	2.93E+03	47.830	6.088	2.77E+06	1.05E+05
Km_Tp	2.50E+05	2.53E+05	45.154	6.090	2.72E+06	1.05E+05
Vmaxc_DTp	1.00E+05	1.01E+05	55.875	6.081	2.92E+06	1.05E+05
Km_DTp	1.00E+08	1.01E+08	55.673	6.081	2.92E+06	1.05E+05
Vmaxc_Gp	1.00E+04	1.01E+04	55.769	6.080	2.92E+06	1.05E+05
Km_Gp	2.00E+05	2.02E+05	55.774	6.081	2.92E+06	1.05E+05
Vmaxc_Sp	6.50E+05	6.57E+05	54.713	5.678	2.90E+06	1.05E+05
Km_Sp	2.00E+05	2.02E+05	55.060	5.811	2.91E+06	1.05E+05
Perchlorate Plasma Binding Parameters						
Vmaxc_Bp	9.50E+03	9.60E+03	54.857	6.417	2.90E+06	1.06E+05
km_Bp	1.10E+04	1.11E+04	54.916	5.590	2.91E+06	1.05E+05
Kunbc_p	1.00E-01	1.01E-01	54.948	5.096	2.91E+06	1.04E+05
Perchlorate Urinary Clearance and Permeability Area Cross Products (L/hr-kg)						
ClUc_p	7.00E-02	7.07E-02	54.047	5.399	2.89E+06	1.05E+05
PAGc_p	8.00E-01	8.08E-01	54.905	5.752	2.91E+06	1.05E+05
PAGJc_p	8.00E-01	8.08E-01	54.905	5.752	2.91E+06	1.05E+05
PATc_p	5.00E-05	5.05E-05	23.273	5.776	2.31E+06	1.05E+05
PADTc_p	1.00E-02	1.01E-02	24.398	5.775	2.33E+06	1.05E+05
PASKc_p	4.00E-01	4.04E-01	3.759	-4.354	1.95E+06	9.50E+04
PARBCc_p	1.00E-01	1.01E-01	3.455	-4.508	1.94E+06	9.49E+04

Notes: AUC Thyroid Concentration using original parameters = 1.88E+06 ng ClO_4^-
AUC Serum Concentration using original parameters = 9.94E+04 ng ClO_4^-

Table 14. Sensitivity Analysis for Physical Parameters in the Male Rat Model at 1.0 mg/kg ClO_4^-

Parameter	Original Parameter Value	1% Increase in Parameter Value	AUC Thyroid Sensitivity Coefficient	AUC Serum Sensitivity Coefficient	Increase in AUC Thyroid ClO_4^- (ng)	Increase in AUC Serum ClO_4^- (ng)
BW	3.00E-01	3.03E-01	-5.944	-0.534	9.81E+06	4.67E+05
Blood Flows [fraction of cardiac output, QCc (L/hr)]						
QCc	1.40E+01	1.41E+01	-0.192	-0.014	9.84E+06	4.69E+05
QTc	1.60E-02	1.62E-02	0.021	NS	9.84E+06	4.69E+05
QSKc	5.80E-02	5.86E-02	-0.085	0.001	9.84E+06	4.69E+05
QGc	1.60E-02	1.62E-02	0.128	0.005	9.84E+06	4.69E+05
QLc	1.70E-01	1.72E-01	0.021	NS	9.84E+06	4.69E+05
QKc	1.40E-01	1.41E-01	-0.234	-0.021	9.84E+06	4.69E+05
QFc	6.90E-02	6.97E-02	0.021	NS	9.84E+06	4.69E+05
Tissue Volumes (fraction of bodyweight)						
Vplasc	4.10E-02	4.14E-02	-7.734	-0.701	9.80E+06	4.66E+05
VRBCc	3.30E-02	3.33E-02	-7.649	-0.691	9.80E+06	4.66E+05
VTtotc	7.70E-05	7.78E-05	-7.841	-0.683	9.80E+06	4.66E+05
VDTc	2.44E-01	2.46E-01	7.606	-0.683	9.87E+06	4.66E+05
VTBc	1.57E-01	1.58E-01	-7.500	-0.682	9.80E+06	4.66E+05
VTc	6.00E-01	6.05E-01	-2.322	-0.683	9.83E+06	4.66E+05
VGc	5.40E-03	5.45E-03	-7.649	-0.685	9.80E+06	4.66E+05
VGJc	1.68E-02	1.70E-02	-7.883	-0.710	9.80E+06	4.66E+05
VGBc	4.10E-02	4.14E-02	-7.628	-0.682	9.80E+06	4.66E+05
VSkc	1.90E-01	1.92E-01	-8.799	-0.829	9.80E+06	4.65E+05
VSkBc	2.00E-02	2.02E-02	-7.606	-0.680	9.80E+06	4.66E+05
VLc	5.50E-02	5.56E-02	-7.628	-0.683	9.80E+06	4.66E+05
VKc	1.70E-02	1.72E-02	-7.628	-0.685	9.80E+06	4.66E+05
VFc	7.40E-02	7.47E-02	-7.585	-0.676	9.80E+06	4.66E+05

Notes: NS = sensitivity coefficient less than 0.001
Original AUC Thyroid Concentration = 9.84E+06 ng ClO_4^-
Original AUC Serum Concentration = 4.69E+05 ng ClO_4^-

Table 15. Sensitivity Analysis for Chemical Specific Parameters in the Male Rat Model at 1.0 mg/kg ClO_4^-

Parameter	Original Parameter Value	1% Increase in Parameter Value	AUC Thyroid Sensitivity Coefficient	AUC Serum Sensitivity Coefficient	Increase in AUC Thyroid ClO_4^- (ng)	Increase in AUC Serum ClO_4^- (ng)
Perchlorate Tissue/Blood Partition Coefficients						
PS_p	3.10E-01	3.13E-01	-7.862	-0.728	9.80E+06	4.66E+05
PR_p	5.60E-01	5.66E-01	-7.649	-0.688	9.80E+06	4.66E+05
PK_P	9.90E-01	1.00E+00	-7.649	-0.688	9.80E+06	4.66E+05
PL_p	5.60E-01	5.66E-01	-7.670	-0.692	9.80E+06	4.66E+05
PG_p	1.80E+00	1.82E+00	-7.905	-0.714	9.80E+06	4.66E+05
PGJ_p	2.30E+00	2.32E+00	-7.883	-0.711	9.80E+06	4.66E+05
PT_p	1.30E-01	1.31E-01	12.911	-0.683	9.90E+06	4.66E+05
PDT_p	7.00E+00	7.07E+00	7.606	-0.683	9.87E+06	4.66E+05
PF_p	5.00E-02	5.05E-02	-7.628	-0.684	9.80E+06	4.66E+05
PSk_p	7.00E-01	7.07E-01	-8.885	-0.846	9.80E+06	4.65E+05
PRBC_p	8.00E-01	8.08E-01	-7.649	-0.691	9.80E+06	4.66E+05
Perchlorate Active Uptake Parameters - Vmaxc (ng/hr/kg BW), Km (ng/L)						
Vmaxc_Tp	2.90E+03	2.93E+03	12.890	-0.683	9.90E+06	4.66E+05
Km_Tp	2.50E+05	2.53E+05	-15.745	-0.682	9.76E+06	4.66E+05
Vmaxc_DTp	1.00E+05	1.01E+05	123.554	-0.687	1.04E+07	4.66E+05
Km_DTp	1.00E+08	1.01E+08	120.784	-0.687	1.04E+07	4.66E+05
Vmaxc_Gp	1.00E+04	1.01E+04	122.062	-0.687	1.04E+07	4.66E+05
Km_Gp	2.00E+05	2.02E+05	122.062	-0.687	1.04E+07	4.66E+05
Vmaxc_Sp	6.50E+05	6.57E+05	120.997	-0.806	1.04E+07	4.66E+05
Km_Sp	2.00E+05	2.02E+05	122.914	-0.641	1.04E+07	4.66E+05
Perchlorate Plasma Binding Parameters - Vmaxc (ng/hr/kg BW), Km (ng/L)						
Vmaxc_Bp	9.50E+03	9.60E+03	122.062	-0.500	1.04E+07	4.67E+05
km_Bp	1.10E+04	1.11E+04	122.062	-0.694	1.04E+07	4.66E+05
Kunbc_p	1.00E-01	1.01E-01	122.275	-0.869	1.04E+07	4.65E+05
Perchlorate Urinary Clearance and Permeability Area Cross Products (L/hr-kg)						
ClUc_p	7.00E-02	7.07E-02	115.031	-1.271	1.04E+07	4.63E+05
PAGc_p	8.00E-01	8.08E-01	122.275	-0.685	1.04E+07	4.66E+05
PAGJc_p	8.00E-01	8.08E-01	122.275	-0.686	1.04E+07	4.66E+05
PATc_p	5.00E-05	5.05E-05	100.969	-0.686	1.03E+07	4.66E+05
PADTc_p	1.00E-02	1.01E-02	120.784	-0.687	1.04E+07	4.66E+05
PASKc_p	4.00E-01	4.04E-01	123.341	-0.567	1.04E+07	4.67E+05
PARBCc_p	1.00E-01	1.01E-01	122.062	-0.687	1.04E+07	4.66E+05

Notes: Original AUC Thyroid Concentration = 9.84E+06 ng ClO_4^-
Original AUC Serum Concentration = 4.69E+05 ng ClO_4^-

The sensitivity of serum and thyroid concentrations to model parameters is not linear. At an *iv* dose level of 1.0 mg/kg, the model prediction of the AUC for serum ClO_4^- concentration is most sensitive to urinary clearance (ClUc_p). A one percent increase in this value, from 0.07 to 0.0707 ng/hr-kg, causes a decrease in AUC serum ClO_4^- concentration from $4.69x10^5$ to $4.63x10^5$ ng, with a sensitivity coefficient of -1.271 (Table 15). Serum concentration is next most sensitive to the rate ClO_4^- unbinds from plasma proteins (Clunbc_p), with a sensitivity coefficient of -0.869 (Table 15).

The predicted AUC for total thyroid concentration at a dose level of 1.0 mg/kg-day is most sensitive to changes in the maximum capacity of the thyroid colloid (Vmaxc_DTp). A one percent increase in this value from $1.00x10^5$ to $1.01x10^5$ ng/hr-kg results in an increase in AUC thyroid concentration from $9.84x10^6$ to $1.04x10^7$ ng (Table 15). However, the AUC thyroid concentration is almost equally sensitive to other parameters of saturable processes, including Vmax, affinity constant (Km) and permeability area cross product (PA) values of other saturable tissues.

With a lower *iv* dose of 0.1 mg/kg, the blood serum concentration remains sensitive to changes in urinary clearance, but demonstrates increased sensitivity to the parameters of saturable compartments and effective partitioning with skin (PSk_p) and red blood cells (PRBC_p). Serum concentration is most sensitive to the maximum capacity for plasma binding (Vmaxc_Bp) at this dose level (Table 13).

At the lower dose level of 0.1 mg/kg, thyroid concentrations show a similar sensitivity to parameters of saturable processes, including plasma binding, permeability area cross products and urinary clearance. Yet the predicted thyroid concentrations at both dose levels (1.0 and 0.1 mg/kg) are most sensitive to a change in Vmax_DTp. The Vmax values of the thyroid were established by fitting thyroid radioiodide and perchlorate uptake from several data sets ranging in three orders of magnitude. The ability of the models to simulate thyroid uptake across several data sets and doses indicates the validity of the estimated thyroid Vmax values.

CONCLUSION

In 1998, the US EPA created a conceptual framework for health risk assessment, which designated the initial perturbation of the hypothalamic-pituitary-thyroid axis (inhibition of iodide uptake in the thyroid) to be the key event leading to subsequent adverse effects, including hypothyroidism, potential thyroid tumors and neurodevelopmental effects. Therefore the dosimetrics presented in this report focused on the relationship of the internal perchlorate dose to the inhibition of iodide uptake in the thyroid. However, the species differences in thyroid up-regulation during drinking water exposure (US EPA, 1998; Merrill *et al.*, 2001b), presented a difficulty in correlating rat dosimetrics to the human.

In all studied drinking water dose groups, male rat TSH levels increased within the first day and T_4 levels decreased. By day 14, T_4 levels returned to control values in all but the 10.0 mg/kg-day dose groups (Yu *et al.*, 2000b). In human volunteers, the sub-chronic drinking water exposures did not show significant changes in TSH or thyroid hormones in the two week study period

(Merrill *et al.*, 2001b). The radioiodide inhibition response in humans after drinking water exposure was more similar to the response from acute dosing in rats than from sub-chronic exposure. The delayed effect in human is attributed to a greater thyroid uptake of iodide due to a larger thyroid and colloid volume and the longer half-life of thyroid hormones in plasma resulting from the presence of TBG.

Up-regulation of thyroid NIS occurs also in the pregnant and lactating rat as a result of drinking water exposure to perchlorate and increased estrogen. However, increased thyroid follicular Vmax values with dose were not required with either of these models, as maternal thyroid anion concentrations are diminished due to the changes in the iodinated hormone and loss of anions to the fetus, urine and milk.

Ratios of internal dosimetrics reveal that pregnant and lactating rats have significantly higher AUC serum concentrations than male rats at low doses (<1.0 mg/kg-day). Since plasma perchlorate levels at doses higher than 1.0 mg/kg-day were more similar to those of the male rat, it is apparent that the binding to plasma proteins is responsible for the low dose differences. As previously stated, the estrous cycle affects plasma binding of T_4 and, consequently, perchlorate. The increased plasma binding with pregnancy accounts for the change in male to female plasma perchlorate ratios seen between the 0.1 and 1.0 mg/kg-day dose groups.

The sensitivity analysis was performed on two internal dosimetrics, AUCs of thyroid and serum concentrations, from acute *iv* dosing in the male rat. AUC thyroid perchlorate concentrations are most sensitive to changes in the maximum capacity of the thyroid colloid. This value was well substantiated, through fitting thyroid uptake across several doses and data sets in the adult rat models, but not in the human or fetal and neonate rat models, as thyroid perchlorate concentrations were not available. Due to the small size of the thyroid, thyroid concentrations are also sensitive to changes in most chemical-specific parameters in the model, especially those describing saturable kinetics (in the thyroid and other saturable tissues). Therefore, the authors do not recommended thyroid perchlorate concentrations as a dosimetric. Serum perchlorate concentrations, on the other hand, are most sensitive to alterations in urinary clearance and plasma binding, parameters that have been well validated through data.

Given time constraints, sensitivity analyses were not run for model predicted percent inhibition of thyroidal iodide uptake. It is likely that further analyses would reveal that the follicular Km for perchlorate has the greatest effect on percent of iodide inhibition in the thyroid. In addition, sensitivity analyses at steady state would provide additional insight as to which dosimetric is least affected by variations in model parameters in a less dynamic situation.

The comparison of male rat to human dosimetrics is useful in evaluating the species differences in perchlorate kinetics. These ratios of dosimetrics from pregnant, lactating and perinatal rats can then be applied to other reproductive stages in humans as well. Thus, the PBPK models can be utilized to improve accuracy in predicting exposures to the most sensitive endpoints, the human fetus and neonate. The dynamics of perchlorate impact on thyroid hormones is yet to be addressed in the models. Ultimately, changes in hormone levels should be used to predict adverse effects. However, as currently presented, the models provide a conservative method for calculating human equivalent exposure doses based on the initial response to perchlorate

exposure. When used in conjunction with the dosimetric ratios, these values could be used to develop drinking water standards that are protective across different life stages.

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PBPK-Derived Internal Dosimetrics

Attachment 2

Table 1. PBPK-Derived AUC Serum Concentration from Drinking Water Exposure (ng/L-H)

Dose (mg/kg-d)	Male Rat	Male Rat (adjusted Vmaxc_Tp)[1]	70 kg Human (4 drinks/day)	70 kg Human (2 drinks/day)	15 kg Human[2] (4 drinks/day)	15 kg Human[2] (2 drinks/day)	Pregnant Rat	Fetal Rat	Lactating Rat	Neonate Rat
0.01	3.21E+04	3.21E+04	1.78E+04	1.77E+04	1.27E+04	1.27E+04	5.10E+04	2.24E+04	5.53E+04	2.77E+04
0.1	1.21E+05	1.21E+05	1.22E+05	1.22E+05	8.70E+04	8.68E+04	1.67E+05	1.14E+05	2.23E+05	1.43E+05
1.0	5.49E+05	5.49E+05	1.07E+06	1.07E+06	7.31E+05	7.31E+05	6.13E+05	3.81E+05	6.51E+05	5.43E+05
3.0	1.46E+06	1.46E+06	3.16E+06	3.16E+06	2.16E+06	2.16E+06	1.56E+06	8.77E+05	1.53E+06	8.54E+05
5.0	2.38E+06	2.38E+06	5.25E+06	5.25E+06	3.58E+06	3.58E+06	2.50E+06	1.37E+06	2.42E+06	1.11E+06
10.0	4.66E+06	4.65E+06	1.05E+07	1.05E+07	7.14E+06	7.14E+06	4.85E+06	2.60E+06	4.63E+06	1.72E+06
30.0	1.38E+07	1.38E+07	3.14E+07	3.14E+07	2.14E+07	2.14E+07	1.43E+07	7.50E+06	1.35E+07	4.13E+06
100.0	4.57E+07	4.58E+07	1.05E+08	1.05E+08	7.12E+07	7.12E+07	4.72E+07	2.47E+07	4.45E+07	1.25E+07

Notes: [1] Used dose-adjusted Vmaxc values for perchlorate in the thyroid follicle (Vmaxc_Tp)
[2] Values were not validated with data

Table 2. PBPK-Derived AUC Thyroid Concentration from Drinking Water (ng/L-H)

Dose (mg/kg-d)	300 g Male Rat	300 g Male Rat (adjusted Vmaxc_Tp)[1]	70 kg Human (4 drinks/day)	70 kg Human (2 drinks/day)	15 kg Human[2] (4 drinks/day)	15 kg Human[2] (2 drinks/day)	Pregnant Rat	Fetal Rat	Lactating Rat	Neonate Rat[2]
0.01	2.97E+05	2.97E+05	1.83E+07	1.83E+07	1.27E+07	1.27E+07	1.13E+06	1.31E+06	4.39E+05	1.96E+05
0.1	2.54E+06	2.54E+06	1.18E+08	1.18E+08	9.16E+07	9.09E+07	8.11E+06	1.65E+07	3.98E+06	2.35E+06
1.0	1.04E+07	1.04E+07	2.65E+08	2.62E+08	2.45E+08	2.39E+08	2.34E+07	3.97E+07	1.63E+07	6.50E+06
3.0	1.42E+07	5.60E+07	2.95E+08	2.94E+08	2.85E+08	2.80E+08	3.41E+07	4.59E+07	2.48E+07	8.12E+06
5.0	1.56E+07	1.17E+08	3.04E+08	3.03E+08	2.96E+08	2.93E+08	4.27E+07	5.67E+07	3.15E+07	9.31E+06
10.0	1.76E+07	2.27E+08	3.14E+08	3.13E+08	3.07E+08	3.05E+08	6.28E+07	6.47E+07	4.74E+07	1.20E+07
30.0	2.24E+07	4.10E+08	3.35E+08	3.35E+08	3.25E+08	3.25E+08	1.41E+08	8.84E+07	1.10E+08	2.18E+07
100.0	3.72E+07	6.10E+08	4.00E+08	4.00E+08	3.70E+08	3.70E+08	4.12E+08	1.66E+08	3.26E+08	5.53E+07

Notes: [1] Used dose-adjusted Vmaxc values for perchlorate in the thyroid follicle (Vmaxc_Tp)
[2] Values were not validated with data

Table 3. PBPK-Derived Peak Serum Concentration from Drinking Water (ng/L)

Dose	Male Rat	Male Rat (adjusted Vmaxc_Tp)[1]	70 kg Human (4 drinks/day)	70 kg Human (2 drinks/day)	15 kg Human[2] (4 drinks/day)	15 kg Human[2] (2 drinks/day)	Pregnant Rat	Fetal Rat	Lactating Rat	Neonate Rat
0.01	3.93E+04	3.93E+04	2.13E+04	2.30E+04	1.63E+04	1.27E+07	5.84E+04	26818	7.42E+03	2.84E+04
0.1	1.46E+05	1.46E+05	1.59E+05	1.81E+05	1.23E+05	9.09E+07	1.91E+05	134309	2.41E+05	1.45E+05
1.0	8.84E+05	8.84E+05	1.54E+06	1.80E+06	1.19E+06	2.39E+08	9.53E+05	554611	8.84E+05	5.52E+05
3.0	2.54E+06	2.54E+06	4.60E+06	5.40E+06	3.58E+06	2.80E+08	2.62E+06	1.41E+06	2.29E+06	8.69E+05
5.0	4.18E+06	4.18E+06	7.66E+06	9.00E+06	5.96E+06	2.93E+08	4.28E+06	2.25E+06	3.67E+06	1.13E+06
10.0	8.48E+06	8.48E+06	1.53E+07	1.80E+07	1.19E+07	3.05E+08	8.42E+06	4.36E+06	7.14E+06	2.49E+06
30.0	2.53E+07	2.53E+07	4.59E+07	5.39E+07	3.57E+07	3.25E+08	2.50E+07	1.28E+07	2.10E+07	4.24E+06
100.0	8.42E+07	8.42E+07	1.53E+08	1.80E+08	1.19E+08	3.70E+08	8.30E+07	4.23E+07	6.95E+07	1.29E+07

Notes: [1] Used dose-adjusted Vmaxc values for perchlorate in the thyroid follicle (Vmaxc_Tp)
[2] All calculations are for PND 10 in lactating and neonatal rat

Table 4. PBPK-Derived Peak Thyroid Concentration from Drinking Water (ng/L)

Dose (mg/kg-d)	Male Rat	Male Rat (adjusted Vmaxc_Tp)[1]	70 kg Human (4 drinks/day)	70 kg Human (2 drinks/day)	15 kg Human[2] (4 drinks/day)	15 kg Human[2] (4 drinks/day)	Pregnant Rat	Fetal Rat	Lactating Rat	Neonate Rat[2]
0.01	4.10E+05	4.10E+05	1.95E+07	1.97E+07	1.42E+07	1.27E+07	1.35E+06	1.63E+06	4.98E+04	2.02E+05
0.1	3.62E+06	3.62E+06	1.27E+08	1.28E+08	1.03E+08	9.09E+07	1.00E+07	1.41E+07	4.71E+06	2.40E+06
1.0	1.36E+07	1.36E+07	2.73E+08	2.73E+08	2.62E+08	2.39E+08	2.83E+07	4.87E+07	1.91E+07	6.55E+06
3.0	1.70E+07	5.13E+07	2.99E+08	2.99E+08	2.94E+08	2.80E+08	4.36E+07	6.01E+07	3.03E+07	8.20E+06
5.0	1.84E+07	8.59E+07	3.07E+08	3.07E+08	3.03E+08	2.93E+08	5.74E+07	6.52E+07	4.01E+07	9.41E+06
10.0	2.08E+07	1.84E+08	3.17E+08	3.17E+08	3.13E+08	3.05E+08	9.12E+07	7.51E+07	6.40E+07	1.52E+07
30.0	2.88E+07	3.62E+08	3.43E+08	3.44E+08	3.34E+08	3.25E+08	2.25E+08	1.12E+08	1.59E+08	2.22E+07
100.0	5.58E+07	7.17E+08	4.23E+08	4.29E+08	3.96E+08	3.70E+08	6.92E+08	2.41E+08	4.88E+08	5.67E+07

Notes: [1] Used dose-adjusted Vmaxc values for perchlorate in the thyroid follicle (Vmaxc_Tp)
[2] Values were not validated with data

Table 5. PBPK-Derived 24 hr Cumulative Urine from Drinking Water (ng)

Dose (mg/kg-d)	Male Rat	Male Rat (adjusted Vmaxc_Tp)[1]	70 kg Human (4 drinks/day)	70 kg Human (2 drinks/day)	15 kg Human[2] (4 drinks/day)	15 kg Human[2] (2 drinks/day)	Pregnant Rat	Fetal Rat	Lactating Rat	Neonate Rat
0.01	3.00E+03	3.00E+03	7.00E+05	7.00E+05	1.50E+05	1.50E+05	3.37E+03	N/A	1.73E+03	2.58E+02
0.1	3.00E+04	3.00E+04	7.00E+06	7.00E+06	1.50E+06	1.50E+06	3.39E+04	N/A	1.95E+04	4.57E+03
1.0	3.00E+05	3.00E+05	7.00E+07	7.00E+07	1.50E+07	1.50E+07	3.41E+05	N/A	2.73E+05	3.51E+04
3.0	9.00E+05	9.00E+05	2.10E+08	2.10E+08	4.50E+07	4.50E+07	1.02E+06	N/A	8.82E+05	5.95E+04
5.0	1.50E+06	1.50E+06	3.50E+08	3.50E+08	7.50E+07	7.50E+07	1.71E+06	N/A	1.50E+06	7.97E+04
10.0	3.00E+06	3.00E+06	7.00E+08	7.00E+08	1.50E+08	1.50E+08	3.42E+06	N/A	3.03E+06	1.28E+05
30.0	9.00E+06	9.00E+06	2.10E+09	2.10E+09	4.50E+08	4.50E+08	1.03E+07	N/A	9.18E+06	3.18E+05
100.0	3.00E+07	3.00E+07	7.00E+09	7.00E+09	1.50E+09	1.50E+09	3.42E+07	N/A	3.07E+07	9.79E+05

Notes: NA – urinary output not applicable for fetal rats

[1] Used dose-adjusted Vmaxc values for perchlorate in the thyroid follicle (Vmaxc_Tp)

[2] Values were not validated with data

Table 6 PBPK-Derived AUC Serum ClO_4^- Concentration Between 2 and 4 hrs after Acute *iv* Dose (ng/L-hr)

Dose (mg/kg)	Male Rat	Pregnant Rat	Fetal Rat	Lactating Rat	Neonate Rat
0.01	3.63E+04	1.23E+05	3.45E+04	1.51E+04	7.13E+02
0.1	1.45E+05	4.93E+05	5.94E+05	1.05E+05	4.80E+03
1.0	9.99E+05	3.70E+06	1.78E+06	4.10E+05	1.26E+04
3.0	3.04E+06	1.16E+07	5.38E+06	1.78E+06	1.42E+04
5.0	5.09E+06	1.95E+07	9.00E+06	3.36E+06	1.50E+04
10.0	1.02E+07	3.94E+07	1.81E+07	7.37E+06	1.66E+04
30.0	3.08E+07	7.27E+07	5.43E+07	2.34E+07	2.15E+04
100.0	1.03E+08	3.97E+08	1.81E+08	7.98E+07	3.61E+04

Table 7. PBPK-Derived AUC Thyroid ClO_4^- Concentration from *iv* Dose (ng/L-hr)

Dose (mg/kg)	Male Rat	Pregnant Rat	Fetal Rat*	Lactating Rat	Neonate Rat*
0.01	3.55E+05	1.14E+06	4.09E+05	4.28E+04	5.72E+02
0.1	3.52E+06	9.60E+06	4.95E+06	4.64E+05	4.16E+03
1.0	1.36E+07	2.62E+07	2.50E+07	5.28E+06	1.28E+04
3.0	1.68E+07	3.21E+07	3.01E+07	8.34E+06	1.47E+04
5.0	1.82E+07	3.67E+07	3.24E+07	9.49E+06	1.56E+04
10.0	2.11E+07	4.79E+07	3.69E+07	1.20E+07	1.76E+04
30.0	3.12E+07	9.19E+07	5.34E+07	2.15E+07	2.42E+04
100.0	6.58E+07	2.45E+08	1.10E+08	5.46E+07	4.75E+04

Note: * Values were not validated with data

Table 8. PBPK-Derived Peak Serum ClO_4^- Concentration after *iv* Dose (ng/L)

Dose (mg/kg)	Male Rat	Pregnant Rat	Fetal Rat	Lactating Rat	Neonate Rat
0.01	5.89E+04	4.21E+04	1.50E+04	2.31E+04	8.35E+03
0.1	5.25E+05	2.46E+05	1.07E+05	1.31E+05	5.91E+04
1.0	5.34E+06	2.75E+06	6.47E+05	2.06E+06	3.13E+05
3.0	1.61E+07	8.53E+06	1.95E+06	6.88E+06	4.54E+05
5.0	2.70E+07	1.43E+07	3.27E+06	1.17E+07	4.92E+05
10.0	5.40E+07	2.88E+07	6.56E+06	2.38E+07	5.40E+05
30.0	1.62E+08	8.69E+07	1.97E+07	7.22E+07	6.70E+05
100.0	5.41E+08	2.90E+08	6.59E+07	2.42E+08	1.08E+06

Table 9. PBPK-Derived Peak Thyroid ClO_4^- Concentration after *iv* Dose (ng/L)

Dose (mg/kg)	Male Rat	Pregnant Rat	Fetal Rat*	Lactating Rat	Neonate Rat*
0.01	5.25E+05	1.13E+06	1.83E+05	2.59E+05	3.66E+03
0.1	4.20E+06	8.47E+06	1.98E+06	1.49E+06	4.07E+04
1.0	1.40E+07	3.00E+07	9.10E+06	6.00E+06	3.72E+05
3.0	1.70E+07	5.29E+07	1.14E+07	8.55E+06	4.74E+05
5.0	1.86E+07	7.54E+07	1.24E+07	9.80E+06	4.99E+05
10.0	2.19E+07	1.32E+08	1.38E+07	1.27E+07	5.28E+05
30.0	3.45E+07	3.57E+08	1.89E+07	2.48E+07	6.02E+05
100.0	9.75E+07	1.14E+09	3.97E+07	6.94E+07	8.16E+05

Note: * Values were not validated with data

Table 10. PBPK-Derived Cumulative 24 hr Urine ClO_4^- after *iv* Dose (ng)

Dose (mg/kg)	Male Rat	Pregnant Rat	Fetal Rat	Lactating Rat	Neonate Rat*
0.01	2.29E+03	2.01E+03	N/A	2.54E+02	4.22E+01
0.1	2.47E+04	2.33E+04	N/A	3.01E+03	4.64E+02
1.0	2.75E+05	3.01E+05	N/A	7.54E+04	6.55E+03
3.0	8.52E+05	9.57E+05	N/A	4.98E+05	1.03E+04
5.0	1.43E+06	1.62E+06	N/A	1.03E+06	1.14E+04
10.0	2.89E+06	3.28E+06	N/A	2.43E+06	1.30E+04
30.0	8.74E+06	9.95E+06	N/A	8.18E+06	1.82E+04
100.0	2.92E+07	3.33E+07	N/A	2.83E+07	3.67E+04

Notes: Amount ClO_4^- in urine predicted between 240 and 264 hrs during drinking water exposure
* Values were not validated with data

Table 11. PBPK-Derived Percent Inhibition of Thyroid Iodide Uptake after *iv* Dose of ClO_4^- (including percent inhibition in the adult human after 2 wks of DW exposure)

Dose (mg/kg)	% Inhibition in 300 g Male Rat	% Inhibition in Pregnant Rat	% Inhibition in Fetal Rat*	% Inhibition in Lactating Rat	% Inhibition in Neonate Rat*	% Inhibition in 70 kg Human from DW**
0.01	1.5%	3.2%	-129.1%	0.5%	0.4%	2.8
0.1	16.3%	30.1%	27.9%	5.3%	1.3%	23.7
1	74.5%	88.7%	81.2%	62.9%	3.0%	80.2
3	90.0%	93.8%	90.3%	92.8%	3.3%	92.3
5	93.5%	97.0%	90.4%	95.8%	3.1%	95.2
10	96.2%	97.9%	97.9%	97.6%	3.8%	97.4
30	98.1%	98.6%	98.9%	98.5%	6.1%	98.9
100	98.7%	98.8%	99.2%	98.8%	13.4%	99.4

Notes: All calculations are for PND 10 in lactating and neonatal rat
* Values were not validated with data
** PBPK-derived % inhibition in humans after 2 weeks at a corresponding daily DW dose (therefore, 1 mg/kg represents 1 mg/kg/d for the human)

Table 12. PBPK-Derived Lactational and Placental Transfer of ClO_4^- as Percent of Maternal Dose

Dose (mg/kg-day)	% Dose to Pup Transferred through Lactation[1]	% Dose to Fetus through Placental Transfer[2]
0.01	49.9%	35%
0.1	45.8%	35%
1	19.5%	35%
3	10.3%	35%
5	8.0%	35%
10	6.3%	35%
30	5.0%	35%
100	4.6%	35%

Notes: [1]Calculated between 240 and 264 hr during drinking water exposure
[2]Calculated between 480 and 504 hr during drinking water exposure

Statistics for Michaelis-Menten fits for Different Data Sets Represented in Figures 1 through 8

Attachment 3

The following statistical outputs correlate with Figures 1 through 9 in Attachment 1. Outputs were generated by the computer program SigmaPlot 2000 for Windows, Version 6.00 (SPSS Science, Chicago, IL) during the fitting of the Michaelis-Menten equation to data.

Statistical Output for "Figure 1. Up-regulation of Vmax_TP for ClO_4^- transport into thyroid follicle"

Nonlinear Regression

[Variables]
x = col(1)
DepVar0 = col(2)
[Parameters]
a = 100000 '{{previous: 96651.5}}
b = .1 '{{previous: 2.23094e+007}}
[Equation]
f = a*x/(x+b)
fit f to DepVar0
[Constraints]
[Options]
tolerance=0.000100
stepsize=100
iterations=100

R = 0.99859210 Rsqr = 0.99718619 Adj Rsqr = 0.99648273

Standard Error of Estimate = 1743.6802

	Coefficient	Std. Error	t	P		
a	96651.4502	3174.2486	30.4486	<0.0001		
b	22309382.2901		1941543.3650	11.4905	0.0003	

Analysis of Variance:

	DF	SS	MS	F	P	
Regression	1	4309971650.6009		4309971650.6009		1417.5577
	<0.0001					
Residual	4	12161682.7325	3040420.6831			
Total	5	4322133333.3333		864426666.6667		

PRESS = 21326018.2127

Durbin-Watson Statistic = 1.5058

Normality Test: Passed (P = 0.3211)

Constant Variance Test: Passed (P = 0.0600)

Power of performed test with alpha = 0.0500: 1.0000

Regression Diagnostics:

Row	Predicted	Residual	Std. Res.	Stud. Res.	Stud. Del. Res.
1	11456.3978	-2456.3978	-1.4087	-1.4966	-1.9539
2	17695.6493	-195.6493	-0.1122	-0.1270	-0.1102
3	29914.3603	2085.6397	1.1961	1.5236	2.0369
4	55430.6585	-430.6585	-0.2470	-0.3108	-0.2725
5	79022.1064	-22.1064	-0.0127	-0.0393	-0.0340
6	4146.4612	-1246.4612	-0.7148	-0.7217	-0.6701

Influence Diagnostics:

Row	Cook'sDist	Leverage	DFFITS
1	0.1441	0.1140	-0.7009
2	0.0023	0.2191	-0.0584
3	0.7227	0.3837	1.6072
4	0.0282	0.3687	-0.2083
5	0.0066	0.8957	-0.0997
6	0.0050	0.0188	-0.0927

95% Confidence:

Row	Predicted	Regr. 5%	Regr. 95%	Pop. 5%	Pop. 95%
1	11456.3978	9821.7963	13090.9992	6346.6568	16566.1387
2	17695.6493	15429.7164	19961.5821	12350.3726	23040.9259
3	29914.3603	26915.4995	32913.2210	24219.5647	35609.1558
4	55430.6585	52491.0207	58370.2963	49766.8258	61094.4912
5	79022.1064	74440.2276	83603.9851	72356.4374	85687.7754
6	4146.4612	3482.8063	4810.1161	-740.0476	9032.9700

Statistical Output for "Figure 2. Michaelis-Menten fit of the acute male rat AUC for serum ClO_4^- (PBPK-derived) after *iv* injection vs. experimentally determined percent inhibition of radioiodide uptake"

Nonlinear Regression

[Variables]
x = col(1)
DepVar0 = col(3)
[Parameters]
a = 100 '{{previous: 97.6252}}
b = .1 '{{previous: 408622}}
[Equation]
f = 100*x/(x+b)
fit f to DepVar0
[Constraints]
[Options]
tolerance=0.000100
stepsize=10
"stepsize=1
iterations=100

R = 0.99550036 Rsqr = 0.99102098 Adj Rsqr = 0.99102098

Standard Error of Estimate = 3.4090

	Coefficient	Std. Error	t	P
b	408621.8584	46078.9881	8.8679	0.0030

Analysis of Variance:

	DF	SS	MS	F	P
Regression	0	3847.8867	3847.8867	331.1120	(NAN)
Residual	3	34.8633	11.6211		
Total	3	3882.7500	1294.2500		

PRESS = 41.7781

Durbin-Watson Statistic = 1.1450

Normality Test: Failed (P = 0.0146)

Constant Variance Test: Failed (P = <0.0001)

Power of performed test with alpha = 0.0500: 0.8616

Regression Diagnostics:

Row	Predicted	Residual	Std. Res.	Stud. Res.	Stud. Del. Res.
1	7.2572	5.7428	1.6846	1.7282	21.0936
2	24.5424	-0.5424	-0.1591	-0.2015	-0.1657
3	71.0612	-1.0612	-0.3113	-0.4249	-0.3579
4	88.6806	-0.6806	-0.1996	-0.2117	-0.1741

Influence Diagnostics:

Row	Cook'sDist	Leverage	DFFITS
1	0.1565	0.0498	4.8278
2	0.0245	0.3766	-0.1288
3	0.1559	0.4633	-0.3326
4	0.0056	0.1103	-0.0613

95% Confidence:

Row	Predicted	Regr. 5%	Regr. 95%	Pop. 5%	Pop. 95%
1	7.2572	4.8367	9.6777	-3.8584	18.3728
2	24.5424	17.8851	31.1998	11.8138	37.2710
3	71.0612	63.6765	78.4460	57.9375	84.1849
4	88.6806	85.0773	92.2839	77.2490	100.1122

Statistical Output for "Figure 3. Michaelis-Menten fit of acute male rat AUC for thyroid ClO_4^- (PBPK-derived) after *iv* injection vs. experimentally determined percent inhibition of radioiodide uptake"

Nonlinear Regression

[Variables]
x = col(1)
DepVar0 = col(2)
[Parameters]
a = 100 '{{previous: 204.525}}
b = .1 '{{previous: 5.86531e+006}}
[Equation]
f = 100*x/(x+b)
fit f to DepVar0
[Constraints]
[Options]
tolerance=0.000100
stepsize=100
iterations=100

R = 0.95283924 Rsqr = 0.90790261 Adj Rsqr = 0.90790261

Standard Error of Estimate = 10.9177

	Coefficient	Std. Error	t	P	
b	5865312.9216		1773949.9286	3.3064	0.0455

Analysis of Variance:

	DF	SS	MS	F	P
Regression	0	3525.1589	3525.1589	29.5742	(NAN)
Residual	3	357.5911	119.1970		
Total	3	3882.7500	1294.2500		

PRESS = 723.7655

Durbin-Watson Statistic = 1.8746

Normality Test: Passed (P = 0.6091)

Constant Variance Test: Failed (P = <0.0001)

Power of performed test with alpha = 0.0500: 0.4609

The power of the performed test (0.4609) is below the desired power of 0.8000.
You should interpret the negative findings cautiously.

Regression Diagnostics:

Row	Predicted	Residual	Std. Res.	Stud. Res.	Stud. Del. Res.
1	5.0858	7.9142	0.7249	0.7313	0.6587
2	35.3668	-11.3668	-1.0411	-1.3369	-1.7169
3	70.9340	-0.9340	-0.0856	-0.1040	-0.0851
4	75.1594	12.8406	1.1761	1.3724	1.8368

Influence Diagnostics:

Row	Cook'sDist	Leverage	DFFITS
1	0.0095	0.0174	0.0876
2	1.1599	0.3935	-1.3831
3	0.0052	0.3235	-0.0588
4	0.6811	0.2656	1.1045

95% Confidence:

Row	Predicted	Regr. 5%	Regr. 95%	Pop. 5%	Pop. 95%
1	5.0858	0.5030	9.6686	-29.9601	40.1317
2	35.3668	13.5701	57.1634	-5.6492	76.3827
3	70.9340	51.1727	90.6954	30.9625	110.9056
4	75.1594	57.2538	93.0650	36.0720	114.2468

Statistical Output for "Figure 4. Michaelis-Menten fit of the male rat serum AUC from DW ClO_4^- (PBPK-derived) vs. percent inhibition of radioiodide uptake calculated from equation in Figure 2"

Nonlinear Regression

[Variables]
x = col(1)
DepVar0 = col(2)
[Parameters]
a = 100 '{{previous: 97.6252}}
b = .1 '{{previous: 408204}}
[Equation]
f = 100*x/(x+b)
fit f to DepVar0
[Constraints]
[Options]
tolerance=0.000100
stepsize=10
'stepsize=1
iterations=100

R = 0.99934004 Rsqr = 0.99868051 Adj Rsqr = 0.99868051

Standard Error of Estimate = 1.3137

	Coefficient	Std. Error	t	P
b	408204.0569	14873.3463	27.4453	<0.0001

Analysis of Variance:

	DF	SS	MS	F	P
Regression	0	6530.7905	6530.7905	3784.3314	(NAN)
Residual	5	8.6287	1.7257		
Total	5	6539.4193	1307.8839		

PRESS = 10.1606

Durbin-Watson Statistic = 0.3248

Normality Test: Passed (P = 0.2760)

Constant Variance Test: Passed (P = 0.0600)

Power of performed test with alpha = 0.0500: 1.0000

Regression Diagnostics:

Row	Predicted	Residual	Std. Res.	Stud. Res.	Stud. Del. Res.
1	7.2764	0.3523	0.2682	0.2730	0.2460
2	22.8888	0.8132	0.6190	0.7100	0.6697
3	57.5651	0.4609	0.3508	0.4770	0.4367
4	78.3670	-0.6123	-0.4661	-0.5282	-0.4862
5	92.2368	-1.6684	-1.2700	-1.2958	-1.4222
6	97.2457	-2.1148	-1.6098	-1.6143	-2.0866

Influence Diagnostics:

Row	Cook'sDist	Leverage	DFFITS
1	0.0027	0.0350	0.0469
2	0.1589	0.2397	0.3760
3	0.1932	0.4591	0.4024
4	0.0792	0.2211	-0.2590
5	0.0690	0.0394	-0.2882
6	0.0145	0.0055	-0.1554

95% Confidence:

Row	Predicted	Regr. 5%	Regr. 95%	Pop. 5%	Pop. 95%
1	7.2764	6.6443	7.9085	3.8408	10.7119
2	22.8888	21.2354	24.5423	19.1288	26.6488
3	57.5651	55.2769	59.8533	53.4860	61.6443
4	78.3670	76.7791	79.9550	74.6354	82.0987
5	92.2368	91.5661	92.9075	88.7939	95.6797
6	97.2457	96.9948	97.4966	93.8595	100.6319

Statistical Output for "Figure 5. Michaelis-Menten fit of the PBPK-derived male rat AUC thyroid ClO_4^- from DW vs. percent inhibition of radioiodide uptake calculated from equation in Figure 3"

Nonlinear Regression

[Variables]
x = col(1)
DepVar0 = col(2)
[Parameters]
a = 100 '{{previous: 99.9941}}
b = .1 '{{previous: 5.85465e+006}}
[Equation]
f = a*x/(x+b)
fit f to DepVar0
[Constraints]
[Options]
tolerance=0.000100
stepsize=10
"stepsize=1
iterations=100

R = 0.99999996 Rsqr = 0.99999992 Adj Rsqr = 0.99999991

Standard Error of Estimate = 0.0107

	Coefficient	Std. Error	t	P	
a	99.9941	0.0057	17614.6635	<0.0001	
b	5854649.8597		2206.6607	2653.1717	<0.0001

Analysis of Variance:

	DF	SS	MS	F	P
Regression	1	8943.0161	8943.0161	78342532.3849	<0.0001
Residual	6	0.0007	0.0001		
Total	7	8943.0167	1277.5738		

PRESS = 0.0012

Durbin-Watson Statistic = 1.8861

Normality Test: Passed (P = 0.5945)

Constant Variance Test: Passed (P = 0.4597)

Power of performed test with alpha = 0.0500: 1.0000

Regression Diagnostics:

Row	Predicted	Residual	Std. Res.	Stud. Res.	Stud. Del. Res.
1	5.0900	-0.0100	-0.9315	-0.9432	-0.9330
2	31.4798	0.0002	0.0172	0.0236	0.0215
3	65.2600	0.0100	0.9352	1.2668	1.3511
4	88.5273	-0.0173	-1.6209	-1.7732	-2.3464
5	94.0779	-0.0079	-0.7401	-0.8176	-0.7918
6	96.6838	0.0062	0.5784	0.6520	0.6174
7	98.2509	0.0091	0.8472	0.9728	0.9677
8	98.8181	0.0019	0.1733	0.2007	0.1838

Influence Diagnostics:

Row	Cook'sDist	Leverage	DFFITS
1	0.0113	0.0247	-0.1485
2	0.0002	0.4670	0.0202
3	0.6699	0.4550	1.2346
4	0.3094	0.1644	-1.0409
5	0.0737	0.1806	-0.3717
6	0.0575	0.2129	0.3211
7	0.1507	0.2415	0.5461
8	0.0068	0.2537	0.1072

95% Confidence:

Row	Predicted	Regr. 5%	Regr. 95%	Pop. 5%	Pop. 95%
1	5.0900	5.0858	5.0941	5.0635	5.1164
2	31.4798	31.4619	31.4977	31.4482	31.5115
3	65.2600	65.2424	65.2776	65.2285	65.2915
4	88.5273	88.5167	88.5379	88.4991	88.5555
5	94.0779	94.0668	94.0890	94.0495	94.1063
6	96.6838	96.6718	96.6959	96.6550	96.7126
7	98.2509	98.2381	98.2638	98.2218	98.2801
8	98.8181	98.8050	98.8313	98.7889	98.8474

Statistical Output for "Figure 6. Michaelis-Menten fit of the human AUC for serum ClO_4^- on exposure day 2 (PBPK-derived) vs. calculated percent inhibition of radioiodide uptake"

Nonlinear Regression

[Variables]
x = col(1)
DepVar0 = col(3)
[Parameters]
a = 100 '{{previous: 204.525}}
b = .1 '{{previous: 163839}}
[Equation]
f = 100*x/(x+b)
fit f to DepVar0
[Constraints]
[Options]
tolerance=0.000100
stepsize=100
iterations=100

R = 0.97614256 Rsqr = 0.95285429 Adj Rsqr = 0.95285429

Standard Error of Estimate = 5.6711

	Coefficient	Std. Error	t	P
b	163839.2702	29015.1984	5.6467	0.0300

Analysis of Variance:

	DF	SS	MS	F	P
Regression	0	1300.0236	1300.0236	40.4217	(NAN)
Residual	2	64.3231	32.1615		
Total	2	1364.3467	682.1733		

PRESS = 161.0453

Durbin-Watson Statistic = 1.4229

Normality Test: Passed (P = 0.1166)

Constant Variance Test: Failed (P = <0.0001)

Power of performed test with alpha = 0.0500: 0.0000

The power of the performed test (0.0000) is below the desired power of 0.8000.
You should interpret the negative findings cautiously.

Regression Diagnostics:

Row	Predicted	Residual	Std. Res.	Stud. Res.	Stud. Del. Res.
1	12.4184	3.7816	0.6668	0.7091	0.5795
2	37.3018	3.2982	0.5816	0.8526	0.7557
3	74.6566	-6.2566	-1.1032	-1.3678	-3.8077

Influence Diagnostics:

Row	Cook'sDist	Leverage	DFFITS
1	0.0658	0.1158	0.2097
2	0.8357	0.5348	0.8102
3	1.0050	0.3495	-2.7908

95% Confidence:

Row	Predicted	Regr. 5%	Regr. 95%	Pop. 5%	Pop. 95%
1	12.4184	4.1160	20.7209	-13.3562	38.1931
2	37.3018	19.4580	55.1457	7.0726	67.5310
3	74.6566	60.2320	89.0812	46.3111	103.0021

Statistical Output for "Figure 7. Michaelis-Menten fit of human AUC for thyroid ClO_4^- on exposure day 2 (PBPK-derived) vs. calculated percent inhibition radioiodide uptake"

Nonlinear Regression

[Variables]
x = col(2)
DepVar0 = col(3)
[Parameters]
a = 100 '{{previous: 204.525}}
b = .1 '{{previous: 1.07592e+008}}
[Equation]
f = 100*x/(x+b)
fit f to DepVar0
[Constraints]
[Options]
tolerance=0.000100
stepsize=100
iterations=100

R = 0.99480406 Rsqr = 0.98963512 Adj Rsqr = 0.98963512

Standard Error of Estimate = 2.6591

	Coefficient	Std. Error	t	P	
b	107591935.2370		8030810.5171	13.3974	0.0055

Analysis of Variance:

	DF	SS	MS	F	P
Regression	0	1350.2054	1350.2054	190.9592	(NAN)
Residual	2	14.1413	7.0706		
Total	2	1364.3467	682.1733		

PRESS = 43.5207

Durbin-Watson Statistic = 2.8573

Normality Test: Passed (P = 0.3269)

Constant Variance Test: Failed (P = <0.0001)

Power of performed test with alpha = 0.0500: 0.0000

The power of the performed test (0.0000) is below the desired power of 0.8000.
You should interpret the negative findings cautiously.

Regression Diagnostics:

Row	Predicted	Residual	Std. Res.	Stud. Res.	Stud. Del. Res.
1	15.0734	1.1266	0.4237	0.4540	0.3390
2	43.3218	-2.7218	-1.0236	-1.4126	-20.8006
3	66.0625	2.3375	0.8791	1.1311	1.3325

Influence Diagnostics:

Row	Cook'sDist	Leverage	DFFITS
1	0.0305	0.1291	0.1305
2	1.8048	0.4749	-19.7824
3	0.8388	0.3960	1.0790

95% Confidence:

Row	Predicted	Regr. 5%	Regr. 95%	Pop. 5%	Pop. 95%
1	15.0734	10.9630	19.1838	2.9164	27.2304
2	43.3218	35.4372	51.2064	29.4270	57.2165
3	66.0625	58.8628	73.2622	52.5446	79.5804

Statistical Output for "Figure 8. Michaelis-Menten fit of the HEED of ClO_4^- in drinking water derived from serum AUC vs. predicted acute percent inhibition in the rat (determined from Figure 2)"

Nonlinear Regression

[Variables]
x = col(1)
DepVar0 = col(2)
[Parameters]
a = 100 '{{previous: 204.525}}
b = .1 '{{previous: 3.21187e+006}}
[Equation]
f = 100*x/(x+b)
fit f to DepVar0
[Constraints]
[Options]
tolerance=0.000100
stepsize=100
iterations=100

R = 0.99852317 Rsqr = 0.99704852 Adj Rsqr = 0.99704852

Standard Error of Estimate = 2.0220

	Coefficient	Std. Error	t	P	
b	3211873.8889		190351.4186	16.8734	<0.0001

Analysis of Variance:

	DF	SS	MS	F	P
Regression	0	6905.7754	6905.7754	1689.0678	(NAN)
Residual	5	20.4426	4.0885		
Total	5	6926.2179	1385.2436		

PRESS = 32.3115

Durbin-Watson Statistic = 0.9317

Normality Test: Passed (P = 0.1371)

Constant Variance Test: Passed (P = 0.0580)

Power of performed test with alpha = 0.0500: 1.0000

Regression Diagnostics:

Row	Predicted	Residual	Std. Res.	Stud. Res.	Stud. Del. Res.
1	4.7610	2.5091	1.2409	1.2520	1.3515
2	19.7808	3.0600	1.5133	1.7095	2.3721
3	58.6731	-1.1362	-0.5619	-0.7988	-0.7650
4	79.9772	-1.6327	-0.8075	-0.9145	-0.8963
5	93.0796	-0.8488	-0.4198	-0.4275	-0.3895
6	97.5685	-0.3263	-0.1614	-0.1618	-0.1451

Influence Diagnostics:

Row	Cook'sDist	Leverage	DFFITS
1	0.0282	0.0177	0.1813
2	0.8069	0.2164	1.2465
3	0.6514	0.5052	-0.7729
4	0.2363	0.2203	-0.4765
5	0.0068	0.0356	-0.0749
6	0.0001	0.0048	-0.0101

95% Confidence:

Row	Predicted	Regr. 5%	Regr. 95%	Pop. 5%	Pop. 95%
1	4.7610	4.0701	5.4518	-0.4825	10.0044
2	19.7808	17.3631	22.1986	14.0483	25.5134
3	58.6731	54.9788	62.3674	52.2963	65.0500
4	79.9772	77.5375	82.4169	74.2354	85.7190
5	93.0796	92.0983	94.0610	87.7901	98.3692
6	97.5685	97.2070	97.9299	92.3582	102.7787

Statistical Output for "Figure 9. Michaelis-Menten fit of the HEED of ClO_4^- in drinking water derived from thyroid AUC vs. predicted acute percent inhibition in the rat (determined from Figure 3)"

Nonlinear Regression

[Variables]
x = col(3)
DepVar0 = col(4)
[Parameters]
a = 100 '{{previous: 204.525}}
b = .1 '{{previous: 3.83371e+007}}
[Equation]
f = 100*x/(x+b)
fit f to DepVar0
[Constraints]
[Options]
tolerance=0.000100
stepsize=100
iterations=100

R = 0.99999999 Rsqr = 0.99999999 Adj Rsqr = 0.99999999

Standard Error of Estimate = 0.0040

	Coefficient	Std. Error	t	P	
b	38337137.3084		4609.5107	8316.9646	<0.0001

Analysis of Variance:

	DF	SS	MS	F	P
Regression	0	7373.3835	7373.3835	455805644.1327	(NAN)
Residual	5	0.0001	0.0000		
Total	5	7373.3836	1474.6767		

PRESS = 0.0001

Durbin-Watson Statistic = 2.0040

Normality Test: Passed (P = 0.0566)

Constant Variance Test: Passed (P = 0.0600)

Power of performed test with alpha = 0.0500: 1.0000

Regression Diagnostics:

Row	Predicted	Residual	Std. Res.	Stud. Res.	Stud. Del. Res.
1	5.0759	-0.0010	-0.2542	-0.2569	-0.2313
2	31.4761	-0.0010	-0.2407	-0.3148	-0.2844
3	65.2766	-0.0026	-0.6379	-0.8674	-0.8417
4	88.4955	0.0079	1.9537	2.0510	4.6049
5	96.6783	0.0024	0.5969	0.5997	0.5568
6	98.2593	-0.0022	-0.5453	-0.5461	-0.5037

Influence Diagnostics:

Row	Cook'sDist	Leverage	DFFITS
1	0.0014	0.0207	-0.0337
2	0.0705	0.4157	-0.2399
3	0.6386	0.4591	-0.7755
4	0.4294	0.0926	1.4713
5	0.0033	0.0092	0.0537
6	0.0008	0.0026	-0.0258

95% Confidence:

Row	Predicted	Regr. 5%	Regr. 95%	Pop. 5%	Pop. 95%
1	5.0759	5.0744	5.0774	5.0654	5.0863
2	31.4761	31.4695	31.4828	31.4638	31.4884
3	65.2766	65.2696	65.2836	65.2641	65.2891
4	88.4955	88.4924	88.4987	88.4847	88.5063
5	96.6783	96.6773	96.6793	96.6679	96.6887
6	98.2593	98.2587	98.2598	98.2489	98.2696

DEPARTMENT OF THE AIR FORCE
AIR FORCE RESEARCH LABORATORY
WRIGHT-PATTERSON AIR FORCE BASE OHIO 45433

3 October 2001

MEMORANDUM FOR US EPA
NCEA (MD-52)
RTP, NC 27711
ATTN: ANNIE M. JARABEK

FROM: Elaine A. Merrill
AFRL/HEST
Operational Toxicology Branch
2856 G St, Bldg 79
Wright-Patterson AFB, OH 45433-7400

SUBJECT: Consultative Letter, AFRL-HE-WP-CL-2001-0010, Comparison of Internal Dosimetrics Using PBPK Models for Perchlorate-Induced Inhibition of Thyroid Iodide Uptake and Sensitivity Analysis for Male Rat Model

1. This letter describes the use of four physiologically based pharmacokinetic (PBPK) models to develop dosimetric measures of perchlorate pharmacokinetics. The perchlorate exposure scenarios simulated are serum and thyroid perchlorate concentrations and percent inhibition of iodide uptake into the thyroid after *iv* dosing in the male rat. These same dosimetrics were derived from drinking water exposures in male rat and pregnant and lactating rat as well as drinking water exposures in human subjects. Based on dosimetrics in the male rat, human equivalent exposure doses (HEEDs) were predicted.

2. A PBPK model parameter sensitivity analysis was performed on the male rat model with *iv* dosing at 0.1 and 1.0 mg/kg. All PBPK parameters controlling perchlorate kinetics were individually varied by one percent and the effect of each parameter change on the area under the perchlorate concentration curve in the serum and thyroid was used as a measure of model parameter sensitivity.

3. For further information, please contact me by phone: (937) 255-5150 ext. 3195, fax: (937) 255-1474 or e-mail: elaine.merrill@wpafb.af.mil.

Elaine A. Merrill
Operational Toxicology Branch

Attachments
1. Comparison of Internal Dosimetrics Using PBPK Models for Perchlorate Induced Inhibition of Thyroid Iodide Uptake and Sensitivity Analysis for Male Rat Model
2. PBPK-Derived Internal Dosimetrics
3. Statistics for Michaelis-Menten fits for Different Data Sets Represented in Figures 1 through 8

1st Ind, AFRL/HEST 3 October 2001

MEMORANDUM FOR US EPA
ATTN: MS. ANNIE JARABEK

This letter report has been coordinated at the branch level and is approved for release.

RICHARD R. STOTTS, DVM, PhD
Branch Chief
Operational Toxicology Branch
Human Effectiveness Directorate